Wildlife infectious disease dynamics in the context of seasonality and bird migration

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Abstract

The recent increase in emerging infectious diseases that entered human populations from wildlife systems has led to a considerable amount of research into the ecology of wildlife infectious diseases. Avian Influenza (AI) is one of these diseases that circulates in wild bird populations and has the potential to spill-over to poultry and, besides causing significant economic damage, poses a constant threat to human global health. To date it is probably the best studied wildlife disease. However, we still lack a clear understanding of two fundamental aspects in the infection dynamics of AIV: 1) what are the drivers that contribute to the prominent seasonal infection patterns and 2) what roles do migrating birds play in affecting global and local infection dynamics. Those two major questions are the core of the research presented in this thesis.

Seasonality is thought to be a general pattern in infectious disease dynamics and the dynamics of Avian Influenza in wild duck populations seems to be no exception. Little is known, however, on how geographic differences in seasonality impact these dynamics. In Chapter 2 I aimed to characterise seasonality and tried to capture the most relevant dimensions of seasonal variation using remotely sensed images indicative for vegetation growth (Normalized Differenced Vegetation Index or NDVI). Furthermore, I described the global distribution of seasonality and changes therein over the past 30 years. The results indicate, that seasonality patterns have changed on a global scale and in all ecological relevant dimensions i.e. the strength of the seasonal signal, the onset, the duration and amplitude of the growing season. Within all dimensions, geographic patterns of change emerge with notable differences between the northern and southern continents. And while the less seasonal biomes of mainly the southern hemisphere showed a general decrease in the strength of the seasonal signal, the already highly seasonal biomes of the northern hemisphere experienced a further increase in their seasonality. The chapter highlights that global climate change has a diverse effect on seasonality patterns with potentially equally diverse consequences for human and wildlife systems.

Having demonstrated the profound variation of seasonality across the globe, in Chapter 3 I investigated the effect of those geographic variations on the infection dynamics of avian influenza virus in wild duck populations using a North American data base. On a regional scale, the amplitude and duration of
both the observed prevalence of infection and the force of infection derived from a Susceptible-Infectious-Recovered (SIR) model applied to these data, parallel the underlying seasonal dynamic in the biotic environment, based on NDVI. Notably, while all regions experienced a peak of infection in late summer, regions experiencing profound seasonal variation showed higher peaks and a greater number of total infections compared to regions with lower seasonal variation. These findings suggest that a single set of ecological drivers such as birth pulse, host density and the proportion of susceptible adults, may generate vastly different seasonal infection dynamics depending on the environmental context.

Although I appreciate the fact that migration can have a significant influence on local infection dynamics, migration was not included in the explanation of the seasonal patterns shown in Chapter 3. In Chapter 4, I built on a detailed dataset describing AIV infection dynamics in mallard ducks (Anas platyrhynchos) at a small spatial scale over a full annual cycle. During part of the year, the local mallard community consisted out of resident and migratory mallards. By using a SIR modelling framework and a likelihood profile approach I aimed to evaluate the relative importance of five mechanisms that were suggested to contribute to the differential role of migrants and resident ducks. The results revealed, that a mechanism here called ‘the replacement of migrants’, meaning that a constant influx of migrants that are immunological naïve to local circulating AIV strains, was most important to predict the observed prevalence pattern. This highlights the importance of migratory timing and in how far individuals of one population and between populations perform their migratory journey in synchrony. This pattern can also be characterised in terms of the spatial and temporal connectivity between individuals and their populations. Chapter 5 deals with this phenomenon of ‘migratory connectivity’ in more detail and argues, that this concept, which hitherto mainly described the geographic (i.e. spatial) linking of individuals and populations between one life cycle stage and another, can be developed towards an explanatory and predictive framework by explicitly including the temporal along with the spatial dimension of migration. Exemplarily, for one specific process that is shaped by migratory connectivity and the timing of migration – the transmission of parasites and the dynamics of diseases – the Chapter underpins the arguments and quantitatively demonstrates that variations in migration phenology and synchrony yield disease dynamics that significantly differ from a time-aggregated case.
Studying the interface of wildlife disease dynamics and animal migration, researchers are often hampered by the ability to track animals over long distances and across the entire annual cycle. In part this is due to the costs of tracking devices often prohibiting studies requiring high sample sizes needed to account for inter-individual variations and have infected animals included in the sample (since most wildlife disease show relatively low prevalences). In these cases Light level geolocators might be the technology of choice since it provides a low-weight and low-cost tracking device that allows the estimation of positions from recorded light intensity measurements. On the downside, the technique is susceptible to any kind of shading that alters the light perceived by the logger. Thus positions show generally low accuracy and one might challenge their applicability to study detailed migratory behaviour. In Chapter 6 I compare different methods to derive population level movement patterns including data from geolocators analysed with recently developed simple and sophisticated analysis tools. Besides new insights into the migration patterns of Sanderlings (*Calidris alba*) the results show that recent advances in both the tracking devise and the analysis tools allow quantifying population level connectivity patterns and therewith provide evidence that this technology can be used to feed studies on host-pathogen interactions with knowledge on individual and population migratory performance.
Chapter 1

Introduction: Wildlife infectious disease dynamics in the context of seasonality and bird migration

“All entities move and nothing remains still”
- Heraclitus, 401 B.C.

The recent increase in emerging infectious diseases that have entered human populations from wildlife systems has led to a considerable amount of research into the ecology of communicable wildlife diseases. And although many theoretical studies suggested the eminent role of parasites on many biological processes for quite some time (Anderson and May 1979, May and Anderson 1979), we now have empirical evidence of how parasites and diseases influence individuals, their population, community structures and even entire ecosystems (Thomas et al. 1998, Hudson et al. 2002a, Wood et al. 2007). However, the inevitable close association between host and parasite that is interconnected on a temporal, organizational, and spatial scale, poses ongoing challenges to researchers and to our understanding of e.g. infectious diseases in wildlife populations.

Avian influenza in wild birds (see Box 1) is a prime example of an extremely complex host-pathogen system with pronounced temporal, spatial and species variation in infection patterns across the globe (Olsen et al. 2006, Munster et al. 2007). It has been demonstrated that infection dynamics in waterbird communities exhibit a clear seasonal pattern (e.g. van Dijk et al. 2014a) likely attributed to seasonal changes in environmental conditions (Herrick et al. 2013 and references therein). However, there is also increasing evidence, that aquatic and semi-aquatic migratory birds play a crucial role in the spread and the amplification of local transmission of the virus (Altizer et al. 2011). The
research included in this thesis aims to increase our understanding of the
relation between global seasonality and how it affects and interconnects bird
migration and wildlife infectious disease dynamics in general and avian
influenza infections dynamics in particular.

**Seasonality** is a predominant feature on most of our planet and arguably one
of the strongest and most ubiquitous sources of external variation influencing
life on Earth (e.g. Fretwell 1972, Blank 1992). In fact, most organisms live in
seasonal environments and during the course of their life cycle, they may be
faced with cold winters and hot summers, seasons of abundant rain and
seasons characterised by drought. This pattern of variation has undoubted
potential to affect natural selection (Darwin 1859) and, in turn, to yield a great
diversity of life history strategies (e.g. Ricklefs 1977, Stearns 1977). These
evolutionary responses have allowed organisms to exploit the favourable and
cope with the annually recurring unfavourable conditions, for instance by
moving elsewhere, using torpor (hibernation or aestivation) or surviving the
unfavourable period in a less susceptible metamorphic stage. As part of these
strategies, species often partition their annual cycle into periods of “growth”
and “non-growth” (e.g. Conover 1992). Thus populations grow in size during
the course of a breeding season, but then decline outside this period. This can
have multiple knock-on effects across entire ecosystems. For example, the
large-scale synchronous nectar production in flowering plants in spring
provides resources for many species of insects and birds allowing them to
reproduce and become a growing resource for animals higher up in the food
chain. When conditions deteriorate and primary production is reduced or
comes to a complete halt in autumn, increased mortality in animals as well as
plant senescence provide resources for yet other groups of animals, plants and
microorganisms (Morisette et al. 2009).

One area that has received a lot of attention over the past 20 years is the role
of seasonality on the population dynamics of infectious diseases (Altizer et al.
2006). Conspicuous seasonal dynamics that are characterised by predictable
annual peaks and troughs in disease incidence have been demonstrated across
a wide range of infectious diseases in both human and wildlife systems (e.g.
Dowell 2001, Altizer et al. 2006). The general trends in seasonality, such as the
latitudinal trend in the phenology of seasons, have been described many times.
However, to my knowledge we lack a more holistic description of the global
patterns of seasonality in all its ecologically relevant dimensions. Besides phenology, seasonal amplitude, duration of the growing season as well as the strength of the seasonal signal also importantly influence selection processes and ultimately define how the different life forms and the various communities and ecosystems they are part of are distributed across the Earth. In **CHAPTER 2**, I use satellite-derived vegetation images to define and describe global patterns of seasonality as well as how seasonality has been affected by climate change during the last three decades.

The mechanisms that underpin the seasonal dynamics of infectious diseases are increasingly well identified (Altizer et al. 2006). Seasonal variation in the

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**Box 1. Avian Influenza Virus**

Influenza A viruses are one of the most ubiquitous viruses present in the world, affecting humans, livestock and wildlife systems (Stallknecht and Brown 2008). The viruses have been found to infect a wide range of vertebrate taxa, however, wild birds and particularly dabbling ducks (*Anas spp.*), Ruddy Turnstones (*Arenaria interpres*) and gulls (*Larus spp.*) have been identified as the natural reservoir of the virus (Webster et al. 1992, Olsen et al. 2006, Stallknecht and Brown 2008). Influenza viruses belong to the family Orthomyxoviridae and are enveloped single stranded, negative sense RNA viruses. The viruses are classified on the basis of two glycoproteins, the hemagglutinin (HA) and the neurominidase (NA) surface proteins. Almost all combinations of the HA (H1-H18) and NA (N1-N11) antigenic subtypes (e.g. H4N1) have been isolated from wild birds (Fouchier et al. 2005, Olsen et al. 2006, Munster et al. 2007). In most cases, avian influenza viruses are of low pathogenic phenotype, causing only mild diseases. However, subtypes from the HA class H5 and H7 may become highly pathogenic and did cause disease outbreaks with up to 100% mortality in poultry flocks (Alexander 2007). Low pathogenic Avian influenza viruses are transmitted primarily through the faecal-oral route and the virus shedding into water via faeces is likely a very efficient transmission pathway between waterfowl (Webster et al. 1992).
environment often results in changes in biological processes that, in turn, have the potential to alter infection dynamics. As discussed above, the vast majority of animal populations show marked seasonal variation in the timing of birth, resulting in a pulsed influx of naïve (susceptible) individuals into the population, which precedes annual peaks in infection prevalence (Hinshaw et al. 1985, van Dijk et al. 2014a). Furthermore, animals living in temporally dynamic environments often experience large variations in resource availability, which they must allocate among competing morphological and physiological processes. Seasonality, however, varies strongly along latitudinal gradients and thus we would expect significant geographical differences in how species allocate available resources between their life-history stages. For example, the duration of the breeding season of many northern hemisphere organisms contracts with greater proximity to the Arctic, thus animals must adjust the rate of energy directed towards reproduction according to the time available (Baker 1939, MacArthur 1964, Wyndham 1986). In CHAPTER 3, I test the hypothesis that regions with higher degrees of seasonality also experience more pronounced disease epidemics. This was accomplished using an extensive, continent-wide surveillance database for North American dabbling ducks that reported avian influenza virus infection rates for 51,903 individuals over a 6-year period (Squires et al. 2012).

The immune system is thought to play a key role in seasonally changing susceptibilities of individuals to infection. This is partly the consequence of trade-offs that exist between allocating existing resources between maintenance of the immune system and other resource-craving activities such as moulting, fattening, or thermoregulation during winter (Sheldon and Verhulst 1996, Norris and Evans 2000). Long-distance migration in birds is another resource-needing activity that is likely to affect immune capacity and ultimately influence pathogen dynamics in host populations. Migration itself has evolved as a response to ameliorate seasonal variations in resource abundance (Dingle 1980, Alerstam et al. 2003, McKinnon et al. 2010). Billions of animals of various taxa migrate annually, often flying substantial distances to reach breeding locations and then returning to non-breeding locations afterwards (Wilcove 2008). Such efforts are accompanied by very high energetic demands and substantial morphological and physiological modifications that are known to modify their immune system, making long-distance migrants more susceptible to infection during the migratory period.
Moreover, throughout their journey, these birds may experience highly diverse and disparate locations, which potentially exposes them to a greater diversity of pathogens (Waldenström et al. 2002). Migrants are also thought to facilitate the long-distance dispersal of these pathogens, thus playing a significant role in pathogen dynamics (Altizer et al. 2011). Many migrants also aggregate in very dense groups at stop-over locations en route, which can enhance parasite transmission and result in an infection prevalence that substantially exceeds that found during other stages of their annual cycle. One prominent example of this is the aggregation of Ruddy Turnstones (Arenaria interpres) feeding on horseshoe crab eggs in Delaware Bay during spring migration (Krauss et al. 2010). These intense aggregations result in extremely high levels of Avian Influenza virus transmission and prevalence. In CHAPTER 4 I use a modelling approach to investigate the potential differential effect of migrant and resident birds on Avian Influenza virus dynamics. The chapter is built on an elaborate, small scale, avian-influenza virus infection surveillance study within a major host species, the Mallard (Anas platyrhynchos) (van Dijk et al. 2014a). In this system, long-distance migrants temporarily mix with a resident population, which provides a prime opportunity to investigate their relative roles in the dynamics of the observed local infection.

The Mallard study provides a prime example of the potential for intraspecific differences in migratory behaviour affecting species-wide infection rates. However, many populations of the same or different species share the same migratory flyways creating huge potential for all sorts of interactions with potential effects on host-pathogen dynamics. The likelihood of this occurring depends importantly on the coincidence of their migration timing. Temporal patterns of migration, migration phenology, can vary substantially between species and populations. At one extreme, all individuals in a population may migrate at the same time (e.g. Orell et al. 2007, Stanley et al. 2012), while at the other extreme, individuals within a given population migrate separately. Specific examples of asynchronous migration include differential migration (e.g. Cristol et al. 1999, Colbeck et al. 2013), where (age-, sex-, or family-) subgroups of a population migrate at different times, or partial migration, where some individuals migrate while others remain resident (e.g. Smith and Nilsson 1987). Obviously, those differences can profoundly influence the dynamics of pathogen levels within migratory hosts and also infection rates within the interacting communities (Altizer et al. 2006, Møller and Szep 2011).
However, the potential for direct or indirect interactions among migratory birds require knowledge of their connectivity. **Migratory connectivity** describes the “geographic linking of individuals and populations between one life cycle stage and another” (Webster et al. 2002). It is a concept that can importantly contribute to answering a range of both fundamental and applied questions relating to the consequences of different migration strategies. The migratory connectivity concept, however, has thus far been mainly descriptive and lacking consideration of the potential effect of temporal patterns. **CHAPTER 5** argues that the migratory connectivity concept provides a valuable explanatory and predictive framework to examine the influence of long-distance migrants on a range of different ecological processes (Bauer and Hoye 2014), one being the dynamics of disease transmission among birds. This requires explicit consideration of temporal influences on inter-individual interactions within the migratory connectivity framework. Particularly in the context of disease dynamics, most consequences not only depend on which sites are used, i.e. the spatial dimension of migratory connectivity, but importantly also on when and for how long these are used.

Although migratory birds are recognised as one of the most important hosts and notably vectors of infectious diseases (e.g. Hubalek 2004, Altizer et al. 2011), one of the major impediments in clarifying the sites of infection and transmission has been in mapping their movements. This is particularly pertinent to long-distance migrants, as their vast movements make it difficult to follow individuals and populations year round. This problem is further exacerbated by the need to have a large sample size of individual movement data to overcome the inherent inter-individual variation in migratory movement (Hoye et al. 2011, Hoye et al. 2012b). Fortunately, advances in **bird tracking technologies** offer significant potential to overcome these constraints. Miniaturized GPS (global positioning system) devices can be used to determine locations of individuals with increasing accuracy (Bridge et al. 2011), and new satellite technology (e.g. the International Cooperation for Animal Research Using Space – ICARUS – project) will provide global tracking data for a substantial number of understudied species (Wikelski et al. 2007). However, light-level archival tags, also called geolocators, are currently the smallest and cheapest means to obtain location data for long-distance movements (Bridge et al. 2011). The principal behind geolocator tracking is simple. The tags record light levels at regular time intervals and after a geolocator has been attached
to a bird for a desired time period, the retrieval of its stored data can be used to infer solar positions that, in turn, can be used to estimate geographic locations (Hill 1994, Ekström 2004). Estimates of geolocator position are susceptible to any shading that affects the light intensity received by the sensor of the tag (Lisovski et al. 2012) resulting in much larger potential errors, compared to satellite-based tracking (e.g. Phillips et al. 2007, Fudickar et al. 2012, Lisovski et al. 2012). However, recent advances in the design of geolocator devices and improvements in data processing methods (e.g. Lisovski and Hahn 2012, Wotherspoon et al. 2013) may improve our knowledge of the yet understudied interactions of migratory birds such as the degree of migratory connectivity along the flyway. This information will also allow us to infer population-level phenomena based on individual behaviour. Such knowledge is critical for improving our predictive modelling of migratory connectivity and its consequences and applications for e.g. disease transmission and conservation planning (Holdo and Roach 2013). In **CHAPTER 6** I have studied inter-individual variation of movements within a migratory population of Sanderlings (*Calidris alba*). This was done by employing new-generation geolocators to determine individual movement tracks throughout the year and to also compare different geolocator analysis methods and their suitability to infer population level movement patterns and the identification of crucial areas for conservation.
Shifting seasonality in a changing world

Seasonality is a predominant feature of our planet and has significant influence on all levels of life. Recent climate change has changed temperature and precipitation patterns across the globe and thus the basic mechanisms driving seasonal environmental variation. We characterized seasonality aiming at capturing the ecologically most relevant dimensions of seasonal variation using remotely sensed images indicative for primary productivity or vegetation growth (Normalized Differenced Vegetation Index or NDVI), followed by describing the global distribution of seasonality and changes therein over the past 30 years. The results indicate that seasonality patterns have changed on a global scale and in all ecologically relevant dimension i.e. strength of the seasonal signal and onset, duration and amplitude of primary productivity. Within all dimensions, geographic patterns of change emerge with notable differences between the northern and southern continents. And while the less seasonal biomes of mainly the southern hemisphere showed a general decrease in the strength of the seasonal signal, the already highly seasonal biomes of the northern hemisphere experienced a further increase in seasonality. The results therewith highlight that global climate change has a diverse effect on seasonality patterns with potentially equally diverse consequences for human and wildlife systems.
Main text

Seasonality is a predominant feature on our planet and attributed to the tilt of the earth. The resulting annually alternating bias in exposure of the hemispheres to the sun leads to concomitant changes in temperature and precipitation regimes. Since those are the major factors influencing life (Franks et al. 1990, Menzel et al. 2005), seasonality has significantly shaped the look of our world, for instance producing a diversity of distinct habitat types, often referred to as biomes. Examples of these include the tundra around the two poles, characterized by almost year-long snow cover and biological productivity within a very short summer period only, the northern-hemisphere, temperate-deciduous forests exhibiting the highest (within-year) seasonal variations in primary productivity of all terrestrial habitats, and the equatorial evergreen and only slightly varying tropical rainforests. Seasonality not only affects plant life but representatives of all taxa, including their cascading interactions. For example, the large-scale synchronous nectar production in flowering plants during spring provides resources for many species, especially insects but also birds and lizards, allowing them to reproduce and to become a growing resource for animals higher up the food chain. When conditions deteriorate after the seasonal maximum and primary production is reduced or comes to a complete halt in autumn and winter, increased mortality in animals as well as plant senescence provide resources for yet other groups of animals, plants and microorganisms (Figure 1 in Morisette et al. 2009). Animal migration is probably the most appreciated and one of nature’s most overwhelming processes that has evolved in response to seasonal variation in resource abundance (Dingle 1980). Every year, billions of migratory animals travel the planet, transporting nutrients and other organisms, linking otherwise disparate ecosystems worldwide in doing so (Bauer and Hoye 2014). However, many others lack the great powers of locomotion required for migration and have developed other ways of coping with seasonal variations in temperature and resource availability. Hibernation is one such adaptation that is widespread across resident mammalian species (Geiser and Ruf 1995). Also humans are importantly affected by the seasonality of their environment. Locally, agricultural production is importantly governed by the seasons, with global effects on financial markets (Tomek and Peterson 2001, and references therein). Seasonal changes in human behavior and physiology have been recognized since ancient times (Wehr and Rosenthal 1989) and numerous investigations have shown a strong association between the seasons and the
incidence of depression, mania, suicides and suicide attempts (Ackleh and Jang 2007, and references therein). Infectious diseases are also known to vary with seasonal variation in temperature and humidity (Pascual and Dobson 2005) and many allergies are related to seasonal phenologies in the budburst of plants (Nathan et al. 1997).

Anthropogenic land-use changes and burning of fossil fuel have increased greenhouse gas levels in our atmosphere leading to rapid changes in the earth’s climate, both increasing temperatures across the globe and leading to alterations in precipitation patterns (Garcia et al. 2014). Beyond doubt, these ‘global warming’ changes have affected the Earth’s biota (Parmesan 2006). Numerous studies have successfully quantified associated trends of change in seasonal dynamics using remotely sensed vegetation data indicative of primary productivity, such as the Normalized Differenced Vegetation Index (NDVI). These studies not only found changes in the phenology of seasonality (i.e. onset of the reproductive season) but also in the amplitude of the seasonal variation and duration of the reproductive season in the majority of terrestrial, seasonal habitats investigated (e.g. Keeling et al. 1996, Myneni et al. 1997, Tucker et al. 2001, Eastman et al. 2013, Xu et al. 2013). In concert, multiple studies have demonstrated changes in the distribution and abundance of many plants and animal taxa across the globe that appeared biased in directions of observed global warming (Parmesan 2006). Although seasonality has multiple dimensions, i.e. purely phenological dimensions like the onset and the end of seasonal events, but also dimensions describing the shape of the seasonality, like the duration and amplitude of the reproductive season, shifts in phenology and particularly the onset of ‘spring’ have been most commonly reported as an ecological effect of climate change on life (Parmesan 2006, Thackery et al. 2010). The potential effects on life of changes in duration and amplitude, but also the strength of the seasonal signal, are apparently being understudied.

Given the ecological significance of seasonality, its multidimensionality and the lack of a comprehensive overview of how seasonality is changing globally, we characterized seasonality in its most ecologically-relevant dimensions (i.e. onset, duration and amplitude of the reproductive season as well as the strength of the seasonal signal) using remotely sensed NDVI data allowing us to describe the global distribution of seasonality and changes therein over the past 30 years. By using additional information on the spatial distribution of the major biomes, we additionally aimed to reveal patterns of change within these
large communities of flora and fauna forming part of these typical environments.

To quantify the **strength of the seasonal signal** we used wavelet analysis, a powerful tool that is already in use throughout science and engineering and is particularly suitable for ecological time series with short-lived transient components (Cazelles et al. 2008). We used the analysis to first search for significant periodicities within 57.2(lon)x70.8(lat) km grid cells across all terrestrial land with the exception of the Antarctic continent and northern latitudes higher than 75.3 degrees for which data was unavailable. We then extracted the associated annual wavelet power spectrum – indicating the strength of the seasonal signal that is sensitive to the annual amplitude and the temporal consistency of the seasonal time series (see Appendix A.2.2). Following Bradley et al. (2007), the **onset** and the **duration of the growing season**, were extracted from an asymmetric double-sigmoid curve fitted to the NDVI time series of each grid cell showing significant annual periodicity in the wavelet analysis. The **seasonal amplitude** was defined as the 95 percent range of the annual NDVI values (for details on methods and data processing see method section and Appendix A.2.1.1).

Not considering the Antarctic and latitudes higher than 75.3 degrees north, the results revealed that more than 50% of the globe’s surface shows significant seasonal changes in vegetation growth (Figure 2.1), the non-seasonal environments consisting almost exclusively of the largely non-vegetated deserts of the world, including the high-latitude and high-altitude deserts (e.g. Arctic, Himalayas and Andes), as well as large swaths of evergreen tropical and coastal rainforests (e.g. in parts of Amazonas, African rainforests, East Australia). Most of this seasonality is annual with some pockets of bi-annual seasonality occurring mostly in tropical and warmer humid regions of central Africa and south of the Himalayas (Figure 2.1). The strength of the seasonal signal also varies geographically. It is particularly high in northern latitudes, notably the northern temperate region, decreasing towards the south. This general pattern of marked seasonal variation occurring mainly at temperate latitudes in the northern hemisphere and not in the southern hemisphere, is thought to be due to differences in the proximity to the oceans, buffering winter temperatures (Addo-Bediako et al. 2000, Chown et al. 2004). Multiple studies have also demonstrated marked geographical variations in the onset
and the duration of the reproductive season as well as in the seasonal amplitude. A strong correlation exists between the onset of the reproductive season and absolute latitude; enhanced bioproduction within seasonal habitats starts first in areas close to the equator, gradually moving poleward thereafter (e.g. Moulin et al. 1997, Atkinson et al. 2012, Brown et al. 2012, Eastman et al. 2013). Due to the reverse effect at the termination of annual productivity, the duration of the entire reproductive season generally increases from the equator towards the poles (Moulin et al. 1997). This trend is again much stronger on the northern compared to the southern continents. The seasonal amplitude increases from the equator towards the temperate regions of the northern hemisphere, thereafter decreasing again when moving further towards the North Pole and into the taiga and tundra zones. The generally lower amplitude on the southern continents shows no clear patterns across latitudes, although the spatially restricted, strongly seasonal habitats at the

Figure 2.1: a) Spatial distribution of seasonal (annual and bi-annual) and non-seasonal habitats based on seasonal variation in vegetation growth using remotely sensed vegetation data (NDVI). White areas (above 75.3 degrees north and Greenland) were excluded from the analysis. b) The area of annual seasonal, bi-annual seasonal and non-seasonal habitats (horizontal axis) across latitudes (vertical axis). The pie-chart shows the overall proportion of seasonal (annual and bi-annual) and non-seasonal habitats across the globe (excluding the Antarctic and land north of 75.3 degrees north). c) Spatial distribution of the strength of the seasonal signal, defined by the power of the wavelet spectrum from a wavelet analysis using remotely sensed vegetation data (NDVI). d) Running mean and standard deviation of the strength of the seasonality signal across latitudes.
southernmost tips of South America, Africa, Australia and New Zealand also experience markedly larger seasonal amplitudes (Figure 2.1c-d and Moulin et al. 1997).

Similar to the global pattern in the strength of the seasonal signal, changes therein showed pronounced and general differences between the northern and the southern continents: although we observed locally increasing and decreasing patterns on every continent, the vast majority of increases in the strength of the seasonal signal occurred in the northern and thus in the stronger seasonal environments. Conversely, decreases in the strength of the seasonal signal were most noticeable in southern latitudes in areas where the seasonal signal was already relatively weak (Figure 2.2a). Large areas that do not conform to this pattern, such as the south-eastern part of the United States, China and western-central Europe are intensive agricultural areas that experienced a step increase in phosphorus fertilization, allowing year round crop growth and livestock production over the last decades (MacDonald et al. 2011). Most eminent increases in the seasonal signal on the southern continents, contrasting the general trend, were found to occur in areas on the southern edge of the Sahara in Africa, were Brandt et al. (2015) reported an increased greening of the landscape due to an elevation in tree cover caused by more intense rainfalls, and in the central-north of Australia, where anthropogenic driven periodic fire regimes have changed over the last decades (Russell-Smith et al. 2013).

Since many life forms show great adaptations to local conditions, changes within one or multiple dimensions of seasonality may force them to move their distributions in concert. Therefore, we calculated how far (and thus how fast) life would have to minimally travel across the face of the globe to find identical conditions in seasonality in 2011 to those prevailing in 1982. In contrast to the general latitudinal geographical patterns of those seasonal dimensions, at least within the northern continents, these velocities of change revealed vastly different spatial patterns (Figure 2.2). On a global scale, the seasonal onset velocity showed a median speed of 150 km over the last three decades. Besides some geographically isolated regions (e.g. Northwest Africa and Newfoundland) the major hotspots of change were found on the Eurasian continent. Velocities within the seasonal onset of up to 600 km/30 years occurred in Eastern Europe, north of the Black Sea (Ukraine and Russia), as well as within a latitudinal band in Central Russia and two more, north-south
stretching bands in the Russian taiga and tundra. The seasonal duration velocity as well as the seasonal amplitude velocity showed very different regional patterns. Whereas, the seasonal duration velocity (median: 150 km/30 years) showed overlapping hotspots with the seasonal start velocity in Eastern Europe and Central Russia, the seasonal amplitude velocity had both a higher median with 200 km/30 years and revealed notable hotspots on the southern hemisphere. High velocities of change in seasonal amplitude occurred in

Figure 2.2: Changes in the four ecologically most relevant dimensions of seasonality. Upper panel depicts the global distribution and the frequency of changes in the strength of the seasonal signal – power – at locations with significant annual or bi-annual seasonality (grey areas indicate non-seasonal locations). The maps within the lower panel show the seasonal change velocity for start, duration and amplitude of the seasonal dynamic as well as the combination of all three and its frequency. Note, that the color range differs between maps.

Eastern-Central Africa (Mozambique) and the southern parts of the Sahel (north of the bi-annual seasonal habitats that were excluded from the analysis)
as well as in the east of Australia and the east coast of Central South America. Yet other hotspots of velocities of change in seasonality emerged by considering a combination of three dimensions – onset, duration and amplitude. The areas with the biggest combined velocity of more than 1200 km/30 years could be found in New Zealand, Southeast Australia and Central America. Due to its limited size, its geographic isolation (leading to isolation from other seasonal habitats) and the extent of the changes in its seasonality over the past 30 years, New Zealand, Southeast Australia and Central America. For New Zealand, this is due to completely losing its original (1982) seasonal character across its limited range and its geographic isolation, leading to isolation from and long distances to other seasonal habitats. Similarly for South-eastern Australia, which is an “island of seasonality” embedded between the Pacific Ocean and the non-seasonal Australian outback, the velocities of seasonal change are extremely high. Changes in vegetation due to human activities (agriculture) have probably significantly contributed to the locally changed seasonality in this part of the continent. Otherwise, unsurprisingly, regions with high combined velocities importantly overlapped with regions of high one-dimensional velocity with, as expected, a higher median velocity of this aggregated change (260 km/30 years) than found in any of the one-dimensional velocities of change. Accordingly, the combined velocity patterns in Africa and South America reflect the velocity patterns in the seasonal amplitude velocity, whereas the patterns on the northern continents reflect a combination of the seasonal onset and the seasonal amplitude velocities. As a result, major hotspots of change are located in Eastern Europe (Ukraine and West Russia), the United Kingdom the western tip of Alaska and Central Russia.

Biomes represent regions that share climatic regimes and thus possess similar vegetation structures as well as similar patterns of biodiversity (Olson and Dinerstein 2001). Our analysis combining geographic boundaries of the Earth’s major biomes and local changes in seasonal dimensions confirmed that most seasonal biomes experienced an advance in the onset of the reproductive season (Figure 2.3 and Eastman et al. 2013). The effect is strongest in the colder habitats with a mean advance in the onset of the reproductive season of about 9 days within the last three decades. While other studies reported that the northern regions with lowest mean annual temperatures, the tundra and the taiga, are most affected (e.g. Jeong et al. 2011, Brown et al. 2012), we conclude from our analysis, that the biomes with a strong seasonal signal such as the
temperate forests and grasslands, show equally high means and even higher extreme changes in the start of the growing season (97.5% percentile between 17 and 21 days compared to the tundra and the taiga with 11 and 12 days; Figure 2.3). The warmer habitats such as the tropical forests and xeric scrublands experienced the least change and almost no trends, yet, high variations ranging between -15 and 11 days (0.025% and 0.975% percentiles). Also within the 2-dimensional space determined by the other two dimensions of seasonality, seasonal duration and seasonal amplitude, we see patterns of change related to mean annual temperature (Figure 2.3). Regions with low mean annual temperature (below ~0°C) – tundra and taiga - show a trend towards higher seasonal amplitude and also, although less pronounced, an increase in duration of the season. In contrast, regions with higher mean annual temperature – tropical forests, grasslands and savannahs, xeric scrublands, Mediterranean habitats – show almost exclusive negative change in seasonal

Figure 2.3: Left panel: the earth’s 11 major biomes as defined by Olson and Dinerstein (2001). Upper right panel: the change in the start of the vegetation growing season (days) over the mean temperatures for all major biomes. The trend line (with 95% confidence interval) was estimated using a gam model. Error bars around the median show the 20% and 80% percentiles. Lower right panel: the change of seasonality in the major biomes within the seasonal amplitude-seasonal duration space (open circles – 1982; closed circles 2011), where the grey arrows show the direction and the magnitude (normalized to the grid size) based on all data points. The contour lines show the mean annual temperature (1982) of the locations within each grid-cell. Spatial information on biomes were downloaded from: http://maps.tnc.org/gis_data.html
amplitude. The temperate and boreal (i.e. taiga) forests, i.e. the biomes with the highest seasonal amplitude and the strongest seasonal signal, all show an increase in seasonal duration but a slightly contrasting trend in their amplitude; whereas seasonal amplitude has increased in taiga and broadleaf and mixed forests it has slightly decreased in conifer forests (mainly Rocky Mountains, around the European Alps and the southeast of the Himalaya).

Our global characterizations of the changes in the ecologically relevant dimensions of seasonality unequivocally indicate that trends of change differ significantly between locations. Still, geographic patterns in these changes are evident. For example, we show that the northern and strongly seasonal environments tend to become more strongly seasonal whereas areas in the south, already exhibiting weaker seasonality, become less seasonal still. Similarly, across the major biomes of the globe, systematic patterns in the change of seasonality over the past 30 years are evident.

Seasonality has a paramount influence on all levels of life and we already see the consequences of changes within single dimensions of seasonality (Parmesan 2006). For example, shifts in the onset of the reproductive season have been shown to lead to populations altering their timing of seasonal activities (for review see: Parmesan 2006) and have the potential to disrupt the coordination in timing between e.g. the life cycles of predators and their prey, herbivorous insects and their host plants, parasitoids and their host insects, and insect pollinators with flowering plants (Harrington et al. 1999). And while some species’ phenotypic plasticity seems to allow the accurate tracking and coadaptation to phenological shifts (Sparks and Yates 1997, Ozgul et al. 2010), most studies show that interacting species respond differently to environmental changes, leading to so-called phenological mismatches that often involve negative fitness consequences (e.g. Visser and Both 2005, Møller et al. 2008, McNamara et al. 2011, Lane et al. 2012, CaraDonna et al. 2014). Importantly, apart from their focus on the change in a single dimension of seasonality only, these studies have also been fore mostly conducted in the temperature and northern regions of the northern hemisphere. However, as we have shown here, highly diverse changes in seasonality have occurred in almost all terrestrial seasonal habitats across the globe. This finding cautions against extrapolating findings from restricted regions to the rest of the world and constrains our ability to project the consequences of those changes to
global patterns like species distributions, extinction risks and ultimately biodiversity.

**Methods**

For this study we calculated the periodicity and the strength of seasonal vegetation activity and extracted land surface phenology (i.e. onset and termination of the reproductive season) and shape parameters (i.e. seasonal amplitude) from a 30 year continuous remote sensing vegetation index time series (1982-2011). The extracted seasonal features were used to describe the spatial distribution of seasonal environments and analyse the temporal trends over the study period. Data manipulation and analyses were conducted in R Version 3.2 (Team 2008).

**Time series of vegetation activity and snow cover**

We used the newest release (2013) of the satellite imagery based Advanced Very High Resolution Radiometer (AVHRR) Normalized Difference Vegetation Index (NDVI) provided by NOAA/NESDIS Center for Satellite Applications and Research (http://www.star.nesdis.noaa.gov/smcd/emb/vci/VH/vh_ftp.php). The used NDVI time series contains global NDVI observations (except Antarctica and latitudes above 75.3 degrees north) at a 16x16 km spatial resolution and a weekly temporal resolution spanning from July 1981. The weekly values are based on the maximum value for each grid cell from daily images, reducing the influence of e.g. cloud cover. NDVI values for grid cells overlaying large water bodies such as oceans and lakes were removed. Because the presence of snow and ice can significantly affect NDVI values, a normalized difference snow cover index was used to identify when certain grid cells were snow or ice covered after which their NDVI value was set to zero for the respective weekly interval. For this purpose we used the EASE-Grid 2.0 remotely sensed snow-cover data from the northern hemisphere as provided by the National Snow & Ice Data Center at a 25x25 km spatial and weekly temporal resolution until 2011 (Brodzik and Armstrong 2013 updated 2014). Snow cover data was resampled to match the spatial resolution of the NDVI grid. To reduce small scale anomalies and potential spurious values, we aggregated the spatial distribution to a 57.2(lon)x70.8(lat) km grid cell. These aggregated grid cells were used in the below described wavelet analysis. However, during the curve fitting procedure applied to extract the phenological parameters of the annual
Defining seasonal features

*Wavelet analysis:* To define the periodicity of seasonality (bi-annual, annual or higher levels) within each aggregated grid cell, we applied a wavelet analysis to the snow corrected NDVI time series of each aggregated cell. Wavelet analysis was performed using the `wt` function within R package `biwavelet` (Gouhier 2014), using all default settings, including ‘morlet’ as the mother wavelet. The wavelet analysis requires continuous data and any missing NDVI values where estimated using linear interpolation. Apart from two gaps in the NDVI data between week 37 in 1994 and week 2 in 1995 and next week 11 and week 24 in 2004, missing values generally occurred only occasionally. The output matrices for period the power of the wavelet spectrum were used to extract the significant periods (e.g. 1 for annual periodicity/seasonality), based on a regular $X^2$-test, and the associated power for each year of the time series (see Appendix A.2.1). The maximum power values associated with a significant period were used to quantify the strength of the seasonal signal. Periodicity estimates at the start and end of time series are generally fraud with error (‘cone of influence’), and therefore we excluded the first and last year in all further analyses. To extract the temporal trends in power over the entire time period a linear regression was applied across the annual power values ($n = 27$ per grid cell). The predicted values for 1984 and 2010 were used to describe the trend in the strength of the seasonal signal across years. For more details and illustrations see Appendix A.2.1.

*Extracting phenological and shape parameters from NDVI time series:* To be able to extract phenological features of the seasonal variation (i.e. onset and duration of vegetation growing season) we fitted an asymmetric double-sigmoid (ADS) curve to the snow corrected NDVI measurements. This method has previously been satisfactorily applied to describe the seasonal dynamic for different habitat types (Pettorelli et al. 2005, Bradley et al. 2007, Hmimina et al. 2013). The NDVI time series for all 16 grid-cells falling within an aggregated grid-cell were used to fit the ADS curve. The following six steps were applied to all data falling within each aggregated grid-cells showing significant annual periodicity (based on the above described wavelet analysis).
1) A simple cosine function \( y(t) = A \cos(2\pi f t + \phi) + B \) was fitted to the NDVI time series: where \( f \) is the frequency and \( 2\pi f \) the angular frequency (both adjusted to the length of the NDVI time series), \( \phi \) phase, \( A \) the amplitude and \( B \) the mean of \( y(t) \). Optimization of fitting parameters \( A, \phi \) and \( B \) was realized using least square and a Gaussian error distribution. Dates at which \( y'(t)=0 \) (minimum and maximum values) were extracted and used to split the entire time series in sets of years going from ‘winter’ to ‘winter (W-year) and ‘summer’ to ‘summer’ (S-year).

2) An ADS function was fitted to each W-year and S-year separately. The ADS function was computed using the following equation:

\[
NDVI(t) = c1 + \frac{1}{2} * (c2 - \tanh(w1 * (t - \nu)) - \tanh(w2 * (t - \mu)))
\]

where \( \tanh \) is the hyperbolic tangent, \( t \) is the time (week of the year) and \( c1, c2, w1, w2, \nu \) and \( \mu \) are the fitting parameters. In this equation \( c1 \) is the baseline and \( c2 \) the maximum NDVI value, \( w1 \) and \( w2 \) define the slope of the annual increase and decrease of the NDVI variation, while \( \nu \) and \( \mu \) are the dates corresponding to the highest rates of change of NDVI(t). Optimal values for all fitting parameters were estimated using a maximum likelihood routine (mle2 function within R-package bbmle: (Bolker and Team 2014)) using a Gaussian error distribution.

3) A ‘global’ ADS curve was derived by calculating a smooth linear transition from the fitted W-year curve to the S-year curve for periods with a negative slope of the fitted cosine curve and from S-year to W-year for periods with a positive slope of the fitted cosine curve.

4) The minimum and maximum NDVI values for each year and each aggregated grid-cell were extracted as the 5% and 95% percentile of the 16 pooled grid-cell time series, respectively. The seasonal amplitude was calculated by subtracting the minimum value from the corresponding maximum value.

5) Phenological metrics, i.e. the onset of the reproductive season and the termination of the reproductive season, were defined as the date at which the global ADS curve (for each year separately) exceeded 15% of the amplitude (from min value) in spring and fell below 15% of the amplitude (from max value) in autumn. The duration of the reproductive season was derived by calculating the time difference between onset and the termination of the growing season.
6) Temporal trends in the onset and duration of the reproductive season as well as the seasonal amplitude were calculated in the same way as the trends in the strength of the seasonal signal. The velocity of change for a specific seasonal dimension was calculated for each grid-cell as the median distance to the nearest ten grid-cells that showed “similar” values for the respective metric. “Similar” was defined as within 3 days for the start, within 7 days for the duration and within 0.025 NDVI for the amplitude of the growing season. For more details and graphical illustrations of all methods see Appendix A.2.1. The developed R code used to manipulate the raw data, calculate periodicity, extract the wavelet spectrum across years and derive the seasonal metrics can be accessed from: www.github.com/slisovski/Seasonality.

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Chapter 3
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Geographic variation in seasonality and its influence on infectious disease dynamics

Seasonality is a ubiquitous factor that affects many biological processes, including the incidence of infectious diseases. However, seasonality shows profound variation across the globe. Intrinsic properties of host populations, particularly host abundance, immune naivety, and resources available for allocation to pathogen defence, are known to play major roles in the dynamics of infectious diseases. Importantly, each of these properties are influenced by seasonal changes in the environment. Thus, the noted geographic variation in infection dynamics is likely to be underpinned by variation in prevailing seasonal dynamics of the biotic environment. Using avian influenza virus as an example of a globally distributed, intensively investigated, multi-host-pathogen, we investigate the relationship between seasonal epidemics in reservoir communities of ducks and the underlying seasonal dynamic i.e. changes in primary productivity of their habitat, based on remotely sensed vegetation data. On a regional scale, the amplitude and duration of both the observed prevalence of infection in 51,903 individual birds sampled at 136 locations across North America from 2006-2011, and the force of infection derived from a Susceptible-Infectious-Recovered (SIR) model applied to the infection data, parallel the measurements of seasonal dynamic changes in productivity of the biotic environment. Notably, while all regions experienced a peak of infection in late summer, regions experiencing profound environmental variation showed higher peaks and a greater number of total infections compared to regions with lower seasonal variation. These findings suggest that a single set of biological drivers may generate vastly different seasonal infection dynamics depending on the environmental context. Explicit consideration of geographic variation in seasonality may therefore provide a powerful framework for understanding the dynamics of infectious diseases.
Introduction

Conspicuous seasonal dynamics, characterised by predictable annual peaks and troughs in disease incidence, have been demonstrated across a wide range of infectious diseases in humans. Childhood diseases such as measles (Fine and Clarkson 1982) and varicella (Metcalf et al. 2009), waterborne infections (e.g., cholera (Pascual et al. 2002)), aerosol-borne infections such as influenza (Dushoff et al. 2004) and diphtheria (Metcalf et al. 2009), and vector-borne diseases (e.g., malaria (Hoshen and Morse 2004)) have all been shown to exhibit marked seasonal oscillations. Although fewer examples have been described, similar infection dynamics have been demonstrated in wildlife systems, suggesting that seasonality in infectious disease may be a general pattern (Altizer et al. 2006). The mechanisms that underpin seasonal dynamics of infectious diseases are also increasingly well established (Altizer et al. 2006). Seasonal changes in day length and solar intensity typically induce seasonal changes in environmental conditions, such as temperature, rainfall and resource availability. Seasonal changes in these so-called extrinsic environmental drivers often result in changes in intrinsic biological processes that, in turn, have the potential to alter infection dynamics (Hosseini et al. 2004, Koelle and Pascual 2004). For many directly transmitted infectious diseases, transmission depends on the abundance (density) of hosts, their contact rate, and the probability of an infection in the event of making contact (Anderson and May 1979, May and Anderson 1979, Begon et al. 2009). Hence, seasonal variations in these intrinsic biological factors – host density, susceptibility to an infection, and contact rate - have the potential to fundamentally shape infection dynamics (Hosseini et al. 2004, Altizer et al. 2006, Begon et al. 2009). Seasonal changes in the density of susceptible individuals are almost universal. The vast majority of animal populations show marked seasonal variation in the timing of birth, resulting in a pulsed influx of immunologically naïve (i.e. susceptible) individuals into the population (Hosseini et al. 2004, Begon et al. 2009). Such seasonal birth pulses have been shown to both precede annual peaks in infection prevalence in wildlife (e.g. van Dijk et al. 2014a), and to be fundamental to producing these dynamics in empirically validated models (Hosseini et al. 2004, He 2005, Begon et al. 2009).

Seasonal changes in the susceptibility of the standing population may also occur, as a result of resource-based trade-offs. Immune responses to pathogen invasion have the potential to reduce susceptibility; however, these responses require resources that may be required to sustain other individual
requirements, such as growth, reproduction, or survival (e.g. Ilmonen et al. 2000, Prendergast et al. 2004). As a result, immune function, and hence susceptibility, is thought to be subjected to trade-offs with other resource-intensive activities (e.g. Martin et al. 2008). For instance, the energetic demands of reproduction are known to result in lower antibody production and cell-mediated immunity in several bird species (Hillgarth and Wingfield 1997, Moreno et al. 2001).

Critically, the extent of seasonality varies markedly across the globe. At a macro-ecological scale, the amplitude of seasonal variation in day length differs with latitude, being greatest toward the poles and becoming negligible near the equator. As a result, seasonal changes in temperature and resource availability also show broad latitudinal trends, with resource availability having sharper peaks of shorter duration towards the poles and progressively more uniform intra-annual patterns of lower amplitude near the equator. These broad latitudinal patterns of variability in resource availability are further modified by topography, continentally and rainfall, collectively defining the ‘seasonality’ of a region. It is therefore necessary to explicitly consider seasonality, broadly described by two features: seasonal amplitude, describing the severity of the seasonal variation; and seasonal duration, describing the length of the period in which a certain biological process may take place. Given the importance of seasonal variation in host density, susceptibility, and contact rate to infection dynamics (Hosseini et al. 2004, Begon et al. 2009, Altizer et al. 2011), the seasonal dynamics of infectious diseases are likely to show marked differences between regions, according to their degree of seasonality. In particular, the amplitude and duration of infection cycles may be defined by the reverberating influence of seasonality on host abundance, the pulsed influx of susceptible individuals, and on the pathogen defence capabilities of the hosts (Figure 3.1). It has long been recognized that the length of the breeding season of many northern hemisphere organisms contracts with greater proximity to the Arctic (Baker 1939, MacArthur 1964, Wyndham 1986), resulting in a short duration, high intensity birth pulse (Figure 3.1). Similarly, although seasonal birth pulses in humans have been shown in almost all populations worldwide, they tend to be more pronounced in regions with extreme seasonal variation in day length and primary productivity (Becker 1991). Critically, recent theoretical studies have shown that species or locations experiencing seasonal birth pulses of higher amplitude experience greater variation and higher peak prevalence
throughout the year (Dorelien et al. 2013, Martinez-Bakker et al. 2014, Peel et al. 2014). Seasonal modulation of immune function, and hence susceptibility, may also be more distinct in regions experiencing more pronounced seasonality (Adelman et al. 2010). In terms of resource allocation, animals experiencing greater seasonality (short duration, high amplitude seasons) are thought to benefit from their well-defined temporal segregation of different life history stages, stages, such as breeding and moult, to avoid resource trade-offs between resource-demanding stages (Wingfield 2008). Consequently, any potential trade-off between immune function and other resource-intensive activities, such as reproduction, is likely to differ among populations according the degree of seasonality they experience (Figure 3.1).

Marked differences in the seasonal dynamics of infectious diseases between locations have recently been explained by latitudinal variation in intrinsic biological factors across a number of disease systems (Altizer et al. 2004, Hosseini et al. 2004, Martinez-Bakker et al. 2014). However, although seasonal variation in day length exhibits a predictable latitudinal pattern, the time and resources available for biological processes, and hence the degree of seasonality of a region, is often greatly modified by several other geographic factors, including topography and continentality. Here, we directly evaluate the influence of the degree of seasonality on infectious disease dynamics using a globally distributed, intensively investigated, multi-host-pathogen system – avian influenza virus (AIV) in wild birds. Several studies from north-temperate
regions have shown that infections in the reservoir community (dabbling ducks; Webster et al. 1992, Nishiura et al. 2009) show annual epidemics with a distinct seasonal pattern (Hinshaw et al. 1985, Krauss et al. 2004, Munster et al. 2007, van Dijk et al. 2014a). The pulsed entry of naïve juveniles and seasonal changes in host abundance have been proposed as important drivers of these infection dynamics (Hinshaw et al. 1985, Olsen et al. 2006, Munster et al. 2007), which were recently examined on a single site within the north-temperate zone (van Dijk et al. 2014a). Given that such intrinsic drivers are expected to vary with the amplitude and duration of seasonality (Figure 3.1), we hypothesize that the timing, duration, and amplitude of annual epidemics will also vary with the amplitude and duration of seasonality. Specifically, we anticipate more pronounced epidemics in regions experiencing a higher degree of seasonality, and epidemics of lower amplitude and longer duration in regions with more protracted, but lower extent of seasonal amplitude (Figure 3.1). We test this hypothesis using an extensive, continent-wide surveillance database for North America that reported AIV infection in 51,903 individuals from the dabbling duck reservoir sampled between 2006 and 2011. We first classify the degree of seasonality (amplitude and duration) for all sampling locations on the basis of remotely sensed vegetation data, which is indicative of primary productivity (Phillips et al. 2008). This generates regional clusters that display similarity in their degree of seasonality. We then describe regional-scale infection dynamics within these clusters using a simple Susceptible-Infectious-Recovered Model (SIR-model) fit to the observed infection data using a maximum likelihood optimization procedure. Finally, we assess whether these regional infection dynamics match our predictions based on their underlying degree of seasonality.

Methods and Materials

Classification of seasonality

We used the remotely-sensed Normalized Difference Vegetation Index (NDVI) to characterize seasonal variation in primary productivity across the North American continent. Globally, NDVI is recorded weekly at a spatial resolution of 16x16 km, available from the National Oceanic and Atmospheric Administration (NOAA) [ftp://ftp.orbit.nesdis.noaa.gov/pub/corp/scsb/wguo/GVIx/GVIx_VH_16km/NVI]. NDVI values range from -1 to +1. Negative values indicate water bodies, values between 0 and 0.2 indicate almost a complete lack of vegetation and values close to 1 indicate extremely dense
green vegetation cover. We calculated seasonal amplitude ($s_{amp}$) and seasonal duration ($s_{dur}$) for each grid cell in North America based on NDVI values from 2006 to 2011, but excluded those that overlapped with the coastline (to remove artefacts of ocean reflectance). These NDVI values were processed in the following sequence: 1) calculation of a smoothed seasonal curve across the 6 years of aggregated data using the loess function in R package base with span = 0.7; 2) deletion of values from albedo effects of ice/snow cover during winter (in instances where NDVI showed a trough in spring and/or autumn values before the minimum in spring and after the minimum in autumn were removed); 3) derivation of $s_{amp}$ as $\max_{NDVI} - \min_{NDVI}$ from the smoothed seasonal curve; 4) calculation of the duration of the annual productivity peak ($s_{dur}$), defined as the number of days that the smoothed seasonal NDVI curve is greater than or equal to 25% of $s_{amp}$.

**Infection data**

Information on avian influenza virus infection in dabbling ducks sampled across North America was obtained from the NIAID Influenza Research Database http://www.fludb.org (Squires et al. 2012) on 14th February 2014. Due to the low number of samples prior to 2006 and the potential for incomplete reports from 2012 onwards (see Figure A.3.2), we used only records from samples collected from 2006 to 2011. We further filtered the dataset to remove any records from institutions that reported only positive results, or records lacking information on the location of sampling. We then removed all records from birds that were not free-living at the time of sampling (i.e. those found dead or from captive flocks), resulting in a total of 51,903 records from individual birds representing 12 species sampled across 136 sites (Table A.3.1). Seasonal dynamics of these sites was defined by calculating the mean $s_{amp}$ and $s_{dur}$ across all constituent 16 x 16km NDVI cells within a 350-km radius of the centre of the site, reflecting the predominant seasonal parameters of the area. In order to assess the influence of seasonality on infection dynamics on a regional scale we clustered the observed locations on the basis of their respective seasonal parameters ($s_{dur}$ and $s_{amp}$) using a partitioning method ($pam$ in R package cluster (Maechler et al. 2014)) (Figure 3.2). Using k=3 (i.e. searching for 3 clusters) led to the highest homogeneity in sample size between regional clusters (Table A.3.1). Using k>3 lead to additional clusters containing less than 1/10th of the data and sporadic sampling efforts across years.
Regional infection dynamics

To quantify the infection dynamics in each regional cluster, we fitted an SIR (Susceptible, Infectious and Recovered) epidemiological model (after Hénaux et al. 2013) to the observed infection data. Following contact with AIV, a susceptible host becomes infectious and sheds virus for a period of time. Because low-pathogenic AIV is not lethal in ducks (Stallknecht et al. 2007), all birds recover from infection (i.e. stop shedding virus). The model was described by the following set of differential equations:

\[
\frac{dS}{dt} = -\lambda S \quad \text{eqn 1}
\]
\[
\frac{dI}{dt} = \lambda S - \gamma I \quad \text{eqn 2}
\]
\[
\frac{dR}{dt} = \gamma I \quad \text{eqn 3}
\]

where \(\lambda\) is the annual force of infection (FOI) and \(\gamma\) is the recovery rate. Within the annual cycle, FOI was modeled following a Weibull distribution (i.e. increasing followed by decreasing rates) described by both shape and scale parameters. The SIR model assumed a geographically closed population (no immigration or emigration) for each regional cluster, with no birth or mortality and a fixed population size of 4000 individuals reflecting the approximate size of a typical mallard population (Galsworthy et al. 2011). We assumed a recovery rate (\(\gamma\)) of \(1/8\) day\(^{-1}\) (Hénaux et al. 2010, Hénaux and Samuel 2011). The model was integrated across monthly time steps using the ode method in the R Package deSolve (Soetaert et al. 2010). The mle2 maximum likelihood method (R package bbmle (Bolker and Team 2014)) was used to calculate the FOI (\(\lambda_i\)) for each regional cluster (\(i\)) by fitting our SIR model to the monthly (\(t\)) observed prevalence of AIV infection (\(P_{i,t}\)), where \(P_{i,t}\) corresponds to the binomial probability that birds sampled in regional cluster \(i\) in month \(t\) were infected with AIV based on all observations for that cluster and month. The maximum likelihood value for the shape, scale and \(\lambda_i\) of the log-logistic density function were determined by minimizing the negative sum of the binomial log-likelihood values of the predicted monthly prevalence (\(P_{i,t}\)) given the number of AIV-positive ducks (\(I_{i,t}\)) among all ducks (\(N_{i,t}\)) sampled in month \(t\) at cluster \(i\).
Results

Seasonality
We used normalized defiance vegetation index (NDVI) to determine the degree of seasonality with respect to seasonal amplitude ($s_{amp}$ [NDVI]) and seasonal duration ($s_{dur}$ [weeks]). We then used cluster analysis to group the 136 sampling locations across the North-American continent on the basis of similarity in underlying seasonality, which generated three regional clusters (Figure 3.2a).

Figure 3.2: a) Locations of avian influenza virus sampling records from 2006 to 2011 across North America reported on the Influenza research database (www.ird.org). Circle diameter indicates the number of individual samples per location (50-11,370). b) Seasonal parameter space (amplitude and duration, calculated on estimates of primary productivity from the remotely-sensed Normalised Difference Vegetation Index): for each 16x16 km grid cell on the North American continent (grey points); and all sampling locations (red, purple, and blue points; where each color refers to a regional cluster based on the similarity of seasonal amplitude and duration). Locations with seasonal amplitude less than 0.1 are considered a-seasonal and are not shown.
Cluster 3 - representing samples collected in the lower Mississippi basin, along the Gulf coast to the US-Mexico border, and throughout California - was characterized by the lowest average $s_{amp}$ (0.20) and longest $s_{dur}$ (34.2), indicating that these locations all experienced a long productive season with relatively modest inter-seasonal variation in productivity.

Cluster 2 encompassed sampling locations along the upper Mississippi basin, the Great Lakes region, and the central and northern East Coast and was characterized by typical “temperate” dynamics - a high degree of inter-seasonal variation (the highest $s_{amp}$; 0.42) over a relatively long productive season ($s_{dur} = 30.6$). Cluster 1 also had a high degree of inter-seasonal variation ($s_{amp} = 32.6$), however, the length of the productive season was considerably shorter than in the other clusters ($s_{dur} = 21.4$). This cluster predominantly contained samples collected in Alaska, as well as those collected on the Canadian prairies and at Baffin Island.

**Regional infection dynamics**

The SIR model was fitted to infection data collected between May and January, to coincide with the period during which the highest infection peaks of AIV in wild birds have been recorded (Hinshaw et al. 1985, Krauss et al. 2004). Data collected in other months were not included as there were associated with very low sampling effort (Figure A3.1). For cluster 1, no data exists from October onwards, probably as a result of snow cover and low duck abundance during
this period. Furthermore, no data were collected during May in cluster 2. The *scale* parameter of the Weibull distribution was very similar across the three clusters, resulting in a high degree of similarity in the timing of peak prevalence across all three annual force of infection (FOI) curves (Figure 3.4b). Yet, the *shape* parameter of the Weibull distribution (defining the duration of the peak, with low values signifying peaks of extended duration) and the estimated λ (defining the amplitude of the peak) were markedly different across the three

![Figure 3.4: Seasonal dynamics within each of the regional clusters. A) Remotely sensed Normalised Difference Vegetation Index (NDVI) mean across sites and years (2006-2011; solid line) ± standard deviation (shaded area). B) Avian influenza virus (AIV) prevalence in wild dabbling ducks: best fit SIR-model for each regional cluster (solid line) and observed prevalence ± 95% CI (points).](image-url)
clusters leading to distinct annual FOI curves (Figure 3.3). For cluster 3, the FOI curve was characterized by relatively low amplitude ($\lambda = 1.49$) and long duration ($\text{shape} = 4.34$). In contrast, cluster 2 was characterized by an annual FOI curve that had a peak of shorter duration ($\text{shape} = 8.67$), but very high amplitude ($\lambda = 4.74$). Cluster 1 had the shortest peak ($\text{shape} = 10.4$) of intermediate amplitude ($\lambda = 1.84$). The simulated prevalence of infection in each of the regional clusters mirrored both the raw prevalence data, and the amplitude and duration of the estimated FOI dynamic on which each SIR-model was applied (Figure 3.4): long-lasting elevation of prevalence with low amplitude in cluster 3, highly-pulsed, medium amplitude prevalence dynamics in cluster 1, and slightly less pulsed, but high amplitude infection dynamics in cluster 2. Sensitivity analyses of our SIR-model showed that our analyses were robust in response to both the initial prevalence used to start the simulation and the assumed recovery rate, $\gamma$ (Figure A.3.2 & A.3.3).

Discussion

Seasonality is recognized as strongly influencing the dynamics of a wide range of infectious diseases (Fine and Clarkson 1982, Altizer et al. 2006). Although geographic variation in seasonality is also widely recognized, remarkably few studies address the significant effect of geographic variation in seasonality on the dynamics of infectious diseases across large spatial scales. To the contrary, studies that have found variation in the temporal dynamics of infection in different regions have often proposed that non-seasonal mechanisms may underpin the dynamics of infection in these different regions (e.g. Viboud et al. 2006). Based on an ecological understanding of the influence of seasonality on several biological processes, we suggest that a clearer understanding of infectious disease dynamics may be enhanced by considering that the same mechanistic drivers may manifest vastly different infection dynamics depending on the underlying seasonality of a region (Figure 3.1).

A handful of studies have previously suggested that intrinsic drivers may generate a range of infection dynamics, using latitude as a proxy to account for differences in seasonality between regions (e.g. Hosseini et al. 2004, Altizer et al. 2006). Yet, even within North America, seasonal amplitude and duration do not exhibit a linear relationship with latitude (Figure 3.2). Using fine-scale remotely sensed data as a proxy for primary productivity, we were able to directly quantify the degree of seasonality of each of our 136 sampling
locations. By then drawing on an extensive surveillance database we demonstrate a clear link between variation in the degree of seasonality and variation in the dynamics of infection in a wildlife reservoir at a macro-ecological scale. This interaction operates in two dimensions: firstly, the amplitude of the annual infection epidemic was correlated with the amplitude of the inter-seasonal variation in primary productivity; and secondly, the duration of the infection epidemic was correlated with the length of the productive season. Although we cannot disentangle the relative importance of specific intrinsic mechanisms with this dataset, we assume that the pulsed entrance of naïve individuals (Peel et al. 2014) and potential seasonal variation in immune responses (Buehler et al. 2008) are in phase and collectively account for variation in the seasonality (of the biotic environment) and variation in infection dynamics across broad spatial scales.

The tight association between variations in seasonality and infection dynamics indicates that infection dynamics may be predictable on a macro-ecological scale. This association also suggests that the force of infection (likelihood of a susceptible individual becoming infected) for AIV is likely to be highest in regions with high amplitude and medium-to-long-duration seasonal dynamics, such as those experienced in temperate regions (Figure 3.3). Likelihood of AIV infection across the annual cycle is reduced by a contraction of the productive season (as in Arctic regions), and profoundly decreased in regions where there is very little inter-seasonal variation in primary productivity, such as the sub-tropics. Notably, infection dynamics do not appear to be defined by the total annual productivity of a region, but the amplitude and duration of the seasonal pulse. These patterns also have implications for the potential risk of spill-over of AIV from the wild bird reservoir into domestic poultry flocks. Based on our findings, risk of spill-over is likely to be considerably higher during late summer and early autumn in regions with high amplitude and medium-to-long-duration seasonal dynamics.

Previous macro-ecological studies have suggested that predominantly extrinsic factors may underpin spatial distribution of AIV (Fuller et al. 2010, Reperant et al. 2010b, Herrick et al. 2013). For example, by applying ecological niche modelling techniques to the Influenza Research Database on which we based our analyses, Herrick et al. (2013) identified regions with low annual rainfall and low temperatures as having the highest relative predicted risk of AIV infection. Although these studies may appear to contradict our suggestion of the importance of seasonality and its influence on intrinsic biological factors, closer
examination of our findings shows this not to be the case. In fact, if we aggregate our results across the entire annual cycle (as in Fuller et al. 2010, Herrick et al. 2013), the force of infection is clearly higher in colder, more northerly regions of the USA, in keeping with the abiotic conditions that promote both viral persistence (Stallknecht et al. 1990, Brown et al. 2009) and transmission (Lowen et al. 2007) under laboratory conditions. Yet, this lab-based approach neglects the dynamics of AIV within the annual cycle. Critically, when the seasonal dynamics of infection are considered, our continental-scale results, as well as those from single sampling locations (Hinshaw et al. 1985, Olsen et al. 2006, Munster et al. 2007, Altizer et al. 2011, van Dijk et al. 2014a), reveal a vastly different pattern: both prevalence and force of infection show distinct peaks during the warmer months for any given location or region, under conditions that ought to be the least favourable for viral persistence and transmission. By explicitly considering seasonal dynamics and comparing across regions with varying degrees of seasonality our results strongly suggest that intrinsic biological drivers, which vary across broad spatial scales as a result of the amplitude and duration of seasonality, may be of greater importance to the dynamics of AIV than extrinsic drivers, such as the abiotic conditions favouring viral persistence and transmission. While it is tempting to suggest that this could be the case for infectious diseases more generally, it is likely that the relative importance of intrinsic versus extrinsic drivers will differ between host-pathogen systems. For instance, human populations may be partially buffered against inter-seasonal variations in the biotic environment, and social factors such as school terms may dilute the effect of seasonal birth pulses for some diseases. However, our results clearly indicate that the use of ecological niche modelling techniques to map infection prevalence and identify factors contributing to risk may reveal spurious correlations unless the seasonal dynamics of infection are explicitly considered.

The macro-ecological dynamics described here may, of course, be substantially modified on a local scale by seasonal variation in host aggregation behaviour. For instance, animals often congregate at important, species-specific foraging sites. One prominent example is the aggregation of ruddy turnstones (*Arenaria interpres*) feeding on horseshoe crab eggs in Delaware Bay during spring migration. These intense aggregations precipitate extremely high levels of AIV transmission and prevalence (Krauss et al. 2010), during a period with relatively low prevalence at the macro-ecological scale (Fig. 3.4b). Yet, broad-scale differences in intrinsic factors, such as the timing and duration of birth pulses,
have been shown to be critical to the seasonal dynamics of infection, even in systems heavily influenced by seasonal changes in host contact rates (Hosseini et al. 2004). For example, seasonal changes in host aggregation behaviour have been shown to influence the seasonal dynamics of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) (Altizer et al. 2004); however, the seasonal dynamics of this infection, and latitudinal differences in these dynamics, could only be simulated with the inclusion of intrinsic factors (Hosseini et al. 2004). Moreover, seasonal changes to host aggregation behaviour and contact rates are often species-specific: some aggregate to breed while others disperse; migratory animals often aggregate prior to and during migration (Krauss et al. 2010); and yet other species aggregate around food resources during the colder months of the year (e.g. Newton-Fisher et al. 2000). As a result, although seasonal variation in contact rates may modify the dynamics of infection on a local scale, they are unlikely to generate variation in infection dynamics on a continental scale.

Avian influenza virus is arguably one of the best studied wildlife diseases and elaborate surveillance of its occurrence in North American wild birds has produced a unique continental-scale database. By combining the findings of this surveillance with remotely-sensed data reflecting primary productivity across the continent, we have demonstrated that the dynamics of infection differ on the basis of regional variation in seasonality, in line with predictions founded on the seasonality of known intrinsic drivers of infection. Our results therefore provide an important development in the understanding and prediction of infectious disease dynamics. Firstly, these results indicate that understanding the drivers of infection in a seasonal context can be a critical validation for ecological niche methods that rely on spatial variation in temporally aggregated presence- and predictor-variables. Furthermore, these findings clearly illustrate that although infection dynamics may show marked differences across broad spatial scales, these dynamics can be underpinned by the same mechanisms when these mechanisms are themselves influenced by variation in seasonality. Given that AIV is a generalist pathogen transmitted in a density-dependent manner through a vast multi-host community, our study suggests that the application of a seasonal approach, including understanding of spatial variation in seasonality and how this may modify both extrinsic and intrinsic drivers of infection, is likely to yield profound insights into other infectious disease systems.
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The differential role of resident and migrants in avian influenza infection dynamics

Migratory birds are increasingly gaining the central stage when it comes to the spread, the local amplification and the socioeconomic threats of avian influenza viruses (AIV). Although avian migrants are perfect vectors for a variety of pathogens, the mechanism behind the role of migrants in local and global infection dynamics is largely unknown. Here, we built on a detailed dataset describing AIV infection dynamics in Mallard ducks (*Anas platyrhynchos*) at a small spatial scale over a full annual cycle. During part of the year, the local mallard community consisted out of resident and migratory mallards exhibiting disparate infection dynamics. By using a Susceptible-Infectious-Recovered (SIR) modelling framework and a likelihood profile approach we evaluated the relative importance of five potential mechanisms contributing to the differential temporal dynamics of infection among migrants and resident ducks. Those mechanisms were, 1) a pronounced birth pulse, 2) short-term vs. long-term immunity, 3) the proportion of infected, susceptible and recovered migrants entering the local population, 4) differential susceptibility and 5) the replacement of migrants during the peak migratory season. The replacement of migrant function turned out to be the most important mechanism, suggesting that a constant influx of migrants that are immunological naïve to local circulating AIV strains are required to predict the observed prevalence pattern. Besides new insights in the ecology of the host-pathogen system at that location, our results and notably the revealed importance of temporal and spatial demographic patterns, provide guidance for future surveillance studies that aim to increase our understanding of the ecology of AIV infection dynamics in wild bird populations and may ultimately help in decision making processes in global health.
Introduction

High pathogenic avian influenza forms a prime example of an emerging infectious disease with rapid rise in incidences and marked geographic expansion (Alexander 2007). When it comes to the spread, the local amplification and the economic and health threats of avian influenza viruses (AIV), and other zoonotic diseases, migratory birds are increasingly gaining the central stage. Several characteristics of migratory species make them seemingly perfect vectors for a variety of pathogens (Altizer et al. 2011, Bauer and Hoye 2014). During their migratory journey migrants may encounter a broad range of parasite species and strains, thereby increasing the likelihood of transmitting novel parasites to the resident communities they encounter (Waldenström et al. 2002). Moreover, the physiological challenges migrants face during their migration, leading to potential trade-offs with their immune function, may heighten their susceptibility to infection (Buehler et al. 2008). Finally, many migrants aggregate in large numbers at so-called stop-over sites leading to further enhancement of pathogen transmission (Krauss et al. 2010). However, despite the pervasive conceptual support of the role of migrants, clear documented examples of their effect on disease dynamics are surprisingly rare (Altizer et al. 2011). Yet, our ability to design strategies to recognise and mitigate potential threats, and thus minimize the risks of outbreaks in poultry flocks, and potential spill-over to humans, crucially depends on fundamental understanding of the dynamics of infectious diseases in wildlife populations.

In a detailed study on mallard ducks (Anas platyrhynchos) van Dijk et al. (2014a) described AIV infection dynamics at a small spatial scale (i.e. in a duck decoy or catching pond) over a full annual cycle. During part of the year, the local mallard community consisted out of resident and migratory mallards. Using stable isotope ratios in feathers (Altermatt 2010, van Dijk et al. 2014b), van Dijk et al. (2014a) were able to characterise the majority of individuals as either migratory or resident mallards, revealing that the major peak of avian influenza virus infection coincided with the arrival of migratory mallards. However, this observation still allows for several potential mechanisms that could drive local AIV dynamics.

In this study, we built on this unique dataset using a Susceptible-Infectious-Recovered (SIR) modelling framework. We started with a very basic demographic and epidemiological scenario and gradually increased the complexity by adding potential mechanisms that are suggested to drive local AIV infection dynamics in wild birds, hereby considering potentially different,
but non-mutually exclusive, roles of migrants and residents. These mechanisms are:

1 – Birth pulse: Birth is expected to be a major factor influencing seasonal changes to the density of susceptible individuals. The vast majority of animal populations show marked seasonal variation in the timing of birth, resulting in a pulsed influx of immunologically naïve (i.e. susceptible) individuals into the population (Hosseini et al. 2004, Begon et al. 2009). Such seasonal birth pulses have been shown to both precede annual peaks in infection prevalence in wildlife (Hinshaw et al. 1985, Peel et al. 2014) and be fundamental to producing these dynamics in empirically validated models (Hosseini et al. 2004, He 2005, Begon et al. 2009).

2 – Short-term immunity: The vast majority of theoretical AIV infection studies assume long-term or even permanent immunity (e.g. Galsworthy et al. 2011, Nickbakhsh et al. 2014). In fact, the immune response to AIV within the host appears sufficient to attenuate the duration and the intensity of subsequent infections (Fereidouni et al. 2010, Jourdain et al. 2010). However, the relatively weak antibody response may be extremely short lived and detectable for a number of months at most (Kida et al. 1980, Hoye et al. 2011). A recent empirical study demonstrated the appearance of AIV strain-specific antibodies for about 42 days (Curran 2012).

3 – Susceptible migrants: A key feature that put migrants into the spotlight of infectious disease dynamics is the fact that they visit disparate locations throughout their annual cycle (Altizer et al. 2011). In combination with the rather strain specific immune response to AIV infections (Jourdain et al. 2010), migrants may thus be generally susceptible to local AIV strains once they arrive at a new location (Verhagen et al. 2014).

4 – Differential susceptibility: The physiological challenges associated with migratory journeys may result in a trade-off with immune functioning, leading to a reduced immunocompetence in migrants compared to residents (Altizer et al. 2011). These differences in immune status may translate into increased infectiousness in migrants, or the likelihood of migrants becoming infected after contact with an infectious individual.

5 – Replacement of migrants: In the context of infectious disease dynamics, a yet understudied part of migration is the diversity in migratory strategy. At one extreme all individuals of a population may have an identical spatial-temporal pattern in their migration (e.g. Orell et al. 2007, Stanley et al. 2012), while at the other end of the spectrum individuals migrate within a
broad time window and may also not necessarily follow the same route. These differences in migration timing and arrival can have profound effects on the local composition, density and turnover of individuals at breeding, wintering and staging sites, and therewith on the local host-pathogen dynamics (Altizer et al. 2006, Møller and Szep 2011).

The aim of this study is to identify the suitability of these five mechanisms in explaining the observed local AIV infection dynamic.

Methods and Materials

Study Species and Site
Mallards are one of the most common and numerous waterfowl species around the world, with an estimated population size of 19 million individuals (Delany and Scott 2006). The species is also considered to be a main low-pathogenic AIV reservoir in the wild (Webster et al. 1992, Nishiura et al. 2009). Mallards are partially migratory, meaning that throughout their distribution the population consists of both migratory and resident birds. Birds breeding in western Europe (e.g. The Netherlands) are mainly sedentary, and northern breeding birds (e.g. Scandinavia, the Baltic, north-west Russia) migrate in autumn to overwinter between Denmark, northern France and Britain (Scott and Rose 1996).

Sampling
Detailed description of the sampling diagnostic methods can be found in van Dijk et al. (2014a). In short: Mallards were caught at a duck decoy (Payne-Gallwey 1886) located near Oud Alblas (4°42’26”E, 51°52’38”N), The Netherlands. Sampling took place from March 2010 until February 2011. On average, the duck decoy was visited six times per month, capturing approximately 15 individuals per visit, resulting in a total of 1109 AIV samples being collected. For detection of current AIV infection, both cloacal and oropharyngeal samples were taken and analysed within seven days of collection. To determine the origin of the individuals, thus distinguish between migrants and residents, a piece of freshly moulted feather collected from the individuals caught between August and December was analysed using stable hydrogen isotope analysis. The origin of 319 out of 458 individuals could be classified based on feather δ²H.
Modelling

All five modelled mechanisms (“modifications” hereafter) had an identical, background model (Figure 4.1). The background model described mallard demography – birth, death, migration – at the observed location and the basic epidemiological dynamics of AIV.

**Figure 4.1: The structure of the background model and the modifications based on five suggested mechanisms that may drive local AIV infection dynamics.** For the background model, the grey box shows the flowchart of the movement of migrant (M) and resident (R) individuals between the compartments as described by the model equations 1-6. Natural mortality, m, is not depicted but is assumed to occur within all three model compartments and at the same rate for all individuals. The graph below the box shows the general annual demographic dynamics of resident mallards (dashed line) and migratory mallards (solid line) visiting the duck decoy during parts of the annual cycle. For the 1st model modification, the bold dashed line shows the potential dynamics of the resident population with a more pronounced birth pulse. The 2nd model modification assumes a reduced immune rate (σ) and thus a faster loss of immunity against AIV infections. In the 3rd model modification, migrants enter only into the pool of susceptible individuals. In the 4th model modification, the transmission rate β is modelled separately and can have different values for migrants and residents. For the 5th model with replacement of migrants modification, the R(t) curve describes the amount and the shape at which migrants within the infectious and the recovered pool are replaced by new susceptible migrants.
Background model structure

The demography of resident and migrant populations was modelled separately. The background model assumed a resident population of 700 adult individuals, reflecting the approximate number of residents observed at the study site during the study period (van Dijk et al. 2014a). Birth rate ($B(t)$) was modelled for residents only and followed a normal distribution, defined by mean day of birth ($\text{B}_{\text{mean}}$) and its standard deviation ($\text{B}_{\text{sd}}$), which was multiplied by the number of pairs $0.5*N_{\text{pop}}$ and a fixed number of hatchlings per pair ($n_{\text{hatch}}$) in order to derive a daily number of hatchlings that enter the population. All individuals (i.e. residents and migrants) experienced natural mortality at rate $m$. The arrival of migrants at rate $M(t)$ was also modelled following a normal distribution defined by mean arrival date ($\text{A}_{\text{mean}}$) and its standard deviation ($\text{A}_{\text{sd}}$), which was multiplied by the resident population size $N_{\text{pop}}$ and the ratio of migrants to residents ($Pr_{\text{mig}}$). The departure of migrants was modelled by setting the migratory population to 0 at day 92 (1st of April), when migrants were expected to have cleared the area (Cramps & Simmons 1977).

The AIV infection dynamics were modelled using a SIRS model with the components Susceptible (S), Infectious (I) and Recovered (R) (Figure 4.1). AIV transmission occurs mainly through the environment, where infectious birds (I) shed virus potentially causing infection of susceptible birds (S). Because most excreted virus is short-lived in the environment (Domanska-Blicharz et al. 2010), transmission was modelled with a transmission term $\beta$ and was assumed to be density dependent (McCallum et al. 2001). We included a low background transmission rate $\eta$. This allows the occasional re-introduction of the virus in the absence of infectious birds (Galsworthy et al. 2011). Background transmission is a crucial mechanism to enable the persistence of pathogens, particularly within small wildlife communities that are below the critical community size where epidemics cannot be sustained by direct transmission only (Breban et al. 2009). Birds recover from infection at rate $\gamma$ and move from compartment I to R. Loss of immunity occurred at rate $\sigma$, transferring individuals back from R to S. Arriving migrants are allocated across the S, I and R compartments in the same proportions as the actual resident population is distributed across these groups.

These assumptions form the background model that consists of six ordinary differential equations:
Resident population:

\[
\frac{dS_R(t)}{dt} = -\beta(I_R + I_M)S_R + \sigma R_R - \eta S_R - mS_R + B \left( \frac{1}{2} \cdot N_{pop} \right) N_{hatch}
\]  
\text{eq. 1}

\[
\frac{dI_R(t)}{dt} = \beta(I_R + I_M)S_R - \gamma I_R + \eta S_R - mI_R
\]  
\text{eq. 2}

\[
\frac{dR_R(t)}{dt} = \gamma I_R - \sigma R_R - mR_R
\]  
\text{eq. 3}

Migrant population:

\[
\frac{dS_M(t)}{dt} = -\beta(I_R + I_M)S_M + \sigma R_M - \eta S_M - mS_M + M \left( \frac{S_R}{S_R+I_R+R_R} \right) N_{pop} P_r m
\]  
\text{eq. 4}

\[
\frac{dI_M(t)}{dt} = \beta(I_R + I_M)S_M - \gamma I_M + \eta S_M - mI_M + M \left( \frac{I_R}{S_R+I_R+R_R} \right) N_{pop} P_r m
\]  
\text{eq. 5}

\[
\frac{dR_M(t)}{dt} = \gamma I_M - \sigma R_M - mR_M + M \left( \frac{R_R}{S_R+I_R+R_R} \right) N_{pop} P_r m
\]  
\text{eq. 6}

Model modifications

For the 1st, birth pulse modification we divided the resident population into adults (>10 months old) and juveniles (<10 months old) and allowed for differential mortality rates. At the end of the annual cycle (defined at day 92, April 1st) all juveniles were transferred into the pool of adults. The 2nd, short-term immunity modification did not require a structural change of the background model. In the 3rd, migrants enter susceptible modification all migrants enter into the S compartment instead of being distributed across the three compartments. In the 4th, differential susceptibility modification, we allowed for separate transmission rates for residents and migrants (β\(_R\) and β\(_M\), respectively). To model the 5th, replacement of migrants modification, a function describing this replacement was added R(t), which was modelled using a symmetric double logistic function with parameters mean, amplitude, slope and kurtosis (i.e. R\(_{\text{mean}}\), R\(_{\text{amp}}\), R\(_{\text{slope}}\), R\(_{\text{kurt}}\) respectively). Changes in the model equations due to the modifications are shown in Appendix A.4.1.

Model parameterisation

The models aim to describe local AIV infection by finding the optimal demographic and epidemiological parameter values for the various model scenarios. Some parameters were fixed at a value derived from literature or personal observations. For most parameters, only ranges could be defined, and the exact values were sought within these ranges by an optimisation routine (see below). All fixed values and ranges are listed in Table 4.1 and explained below.
In the background model, birth was modelled using a fixed number of 0.63 hatchlings per pair ($N_{\text{hatch}}$). This value ensured a stable population size over time given a natural daily mortality rate ($m$) of $8.63 \times 10^{-5}$, which was based on a life expectancy of 2.27 years for mallards (Schekkerman and Slaterus 2008). For the 1st model modification birth pulse, a larger number of hatchlings was chosen. Mallards are known to produce large clutches with an average clutch size of 9 to 13 eggs (Cramp and Simmons 1977), that potentially result in 4 instead of 0.63 hatchlings per pair that enter the local population (Figure 1). Mortality rates for juveniles $m_j$ were estimated prior to each simulation to ensure stable population size ($N = 700$), taking the $B_{\text{mean}}$, $B_{\text{sd}}$, and $m$ into account. The mean of the time period for autumn migration ($A_{\text{mean}}$) was chosen from 27th Aug. – 30th Nov., depicting the period that migratory mallards may arrive at the wintering grounds in Western Europe.

Table 4.1: The parameters of the background model and model modifications 1, 2, 4 and 5. If the value of a parameter is a single integer, the parameter was hold fixed during the mcmc simulation. All other parameters were optimised within the given range. See methods for references and validation of the defined parameter ranges.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value/Range</th>
<th>Units</th>
</tr>
</thead>
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<tr>
<td>$\beta$</td>
<td>trans. rate</td>
<td>$0.1 \times 10^{-4}$ to $0.4 \times 10^{-3}$</td>
<td>bird$^{-1}$ day$^{-1}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>recovery rate</td>
<td>$1/12$ to $1/3$</td>
<td>day$^{-1}$</td>
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<tr>
<td>$\sigma$</td>
<td>immune rate</td>
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<td>$\eta$</td>
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<td>day$^{-1}$</td>
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<td>day of the year</td>
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<tr>
<td>$B_{\text{sd}}$</td>
<td>standard deviation of birth</td>
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<td>days</td>
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<td>$0.63$</td>
<td>individuals</td>
</tr>
<tr>
<td>$P_{\text{mig}}$</td>
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<td>proportion</td>
</tr>
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<td>day of the year</td>
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<tr>
<td>$A_{\text{sd}}$</td>
<td>standard deviation of arrival of migrants</td>
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<td>days</td>
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<tr>
<td>$m$</td>
<td>mortality rate</td>
<td>$0.315/365$</td>
<td>bird$^{-1}$ day$^{-1}$</td>
</tr>
<tr>
<td>$N_{\text{pulse}}$</td>
<td>number of hatchlings per pair</td>
<td>$4$</td>
<td>individuals</td>
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<td>Days</td>
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</tbody>
</table>
This period resembles the outer boundaries of observed bird migration (Fransson and Petersson 2001, Bakken et al. 2003). The parameter boundaries describing the replacement of migrants \(R(t)\) in the 5th replacement of migrants modification were defined to allow the replacement of individual migrants from the beginning of the migratory period (27th Aug) until mid-October, depicting the peak of migration. Migrants with a current AIV infection were not subject to replacement. An empirical study in swans has shown that AIV infection hampered migration (van Gils et al. 2007b), indicating that those individuals may remain stationary during the course of an infection.

AIV transmission rates \(\beta\) in wildlife populations are largely unknown. We chose a broad range for \(\beta\), making sure that the basic reproduction number of the virus \(R_0\) ranges from about 0.8 to 8 (for \(\beta\) and \(\beta_R\)) and up to 15 for \(\beta_M\). The background transmission \(\eta\) was set to a fixed value of \(\eta = 10^{-5}\) which gives a probability of 1% for a single duck to become infected from outside the duck-decoy in a mean lifetime of 828 days (Galsworthy et al. 2011). The mean recovery rate \(\gamma\) and the immune loss rate \(\sigma\) can vary between host-species, their immunological history and between virus strains (Costa et al. 2010, Fereidouni et al. 2010, Jourdain et al. 2010, Curran 2012). A mild strain may cause longer periods of virus excretion \(\gamma=1/12\) (Kida et al. 1980, Costa et al. 2010, Jourdain et al. 2010), whereas a more severe strain has been shown to have a short generation time of approximately three days \(\gamma=1/3\) (Ng and Higgins 1986, van der Goot et al. 2008, Latorre-Margalef et al. 2009). In all models, except for the 2nd short-term immunity model modification, the immune rate \(\sigma\) was set to a value resulting in a mean loss of immunity within 75 and 730 days. In the 2nd model the immune rate range was changed to allow for short-term immunity with a mean loss of immunity up to 14 days. All parameters, their defined values or ranges and their units are shown in Table 4.1.

Simulation and model fit

To allow demographic and infection patterns to stabilize, all models were run for ten annual cycles. The last cycle was used for comparison with the patterns observed in the field. All possible model combinations \(n = 32\) were written in C++ and compiled as well as integrated using the ode method in R Package deSolve (Soetaert et al. 2010). To estimate parameter values, their relative importance and uncertainties, we used a Markov Chain Monte Carlo (MCMC) simulation with an adaptive Metropolis algorithm implemented in the function
MCMCmod from R Package FME (Soetaert and Petzoldt 2010). The metropolis algorithm optimizes the parameter settings by minimizing the negative sum of the log-binomial densities for each observation using the model prediction as the probability of success. All MCMC simulations were run using 20,000 iterations with an update of the covariate matrix after every 100 iterations. The possible parameter space for all non-fixed parameters was defined as shown in Table 4.1. The last 10,000 iterations were used to describe the posterior distribution of each parameter and to calculate the maximum likelihood value of the best fit for all possible model combinations. Observations for resident birds and migrants were compared separately with the respective model output (prevalence in residents and prevalence in migrants). The number of infected and uninfected individuals with unknown migration status were also included and compared with the pooled predicted prevalence of both residents and migrants. Thus, the negative sum of the log-binomial density consisted of three separate density functions: residents, migrants and unknown (migrants plus residents). To estimate the correlation matrices of the model parameters in relation to the negative log-likelihood, we ran a Monte Carlo Analysis (implemented in function modCRL in R Package FME) that estimated the global effect of each parameter combination within their possible ranges. The modelling code is available at www.github.com/slisovski/Mallard-SIRS-Modeling and the surveillance data has been submitted to the influenza research database www.fludb.org.

Results

The background model in combination with the five different model modifications led to 32 possible scenarios (Figure 4.2). The negative log likelihood values of the best model fit for the 32 scenarios ranged from 113.9 to 321.9 with lower values indicating better model solutions. Although their annual AIV dynamics appeared different, the best fitting four scenarios were within a small negative log-likelihood range of 0.73 points, and could be considered similarly good (Figure 4.3).
Two modifications to the background model were found to generally produce better model fits. The 15 best ranked scenarios all included the 5th replacement

![Graph showing model fits and parameter estimations](image)

Figure 4.2: All model combinations with the negative log likelihood of the model fit. Orange bars indicate scenarios with a single modification on top of the background model. Models with lower negative log likelihood values represent better model solutions. The inclusions of the five model modifications to the background model are shown below the bar plot and a cross indicates that this particular modification was part of the scenario with the associated negative log-likelihood value from above. Below, all estimated parameter values across the 32 scenarios are shown with the 50% (circle) and the 30% and 70% percentiles of the posterior distributions (10,000 iterations). The grey areas between the dashed lines indicate the pre-set parameter ranges. In case of $\beta$ and $\sigma$, the dotted lines indicate the reduced boundaries of the parameter for a certain model modification.
of migrants modification and were strongest in predicting the pronounced and long-lasting prevalence peak during autumn. The replacement of migrant modification was also the best modification in explaining the observed AIV prevalence pattern in isolation with the background model (rank 14, Figure 4.3: Results of the four best fitting model scenarios with similar negative log-likelihood estimates (113.8 – 224.6). The 5th scenario with a slightly higher neg. log-likelihood value of 116.3 is also shown as it presents the two most important model modifications, the 5th replacement of migrants and the 4th susceptibility of migrants. The left column shows which model modifications (1-5: see Fig. 1) were included in the respective scenario. The model prediction (red line) and its 95% CI (grey area) are shown in the second column and on top of the observed AIV prevalence levels (±95% CI). The underlying demographic pattern for each of the five scenarios are shown in the right column.
On top of the replacement of migrant modification, the 4th differential susceptibility modification was present in the seven top ranking scenarios. Amongst those seven models, the 5th best ranked scenario represented only those two (4th and 5th) modifications (Figure 4.3), whereas the other scenarios included one or more of the other modifications too (1st-4th). No eminent pattern was found in the distribution of the other three modifications across the 32 scenarios ranked by the negative log-likelihood (Figure 4.2).

Looking at the best parameter combinations across all 32 scenarios (Figure 4.2), once the transmission rates were modelled separately for migrants and residents, $\beta_M$ was always estimated higher than $\beta_R$. However, except for four scenarios, $\beta_M$ was chosen within the boundaries of $\beta_R$. Only in the relatively poorly fitting scenarios 16-17 and 19-20 that represent the best fitting scenarios without the 5th replacement of migrants modification, $\beta_M$ exceeded the upper boundary $\beta_R$. The recovery rates ($\gamma$) were always at the lower end of the pre-defined parameter range: AIV infected individuals recovering after an average of approximately 12 days. The immune rates ($\sigma$) were highly variable across the different scenarios and thus unrelated to the goodness of fit of the models.

![Figure 4.4: The parameters of the best model fit (matrix diagonal) and the correlation matrix between all parameters. The colour and shape of the ellipsoids scale with the correlation coefficient from -1 to 1.](image-url)
However, in the 2nd model with short-term immunity modification, the fitting procedure resulted in higher values for $\sigma$ and in 11 out of 16 scenarios, those values were higher than they would have been in the absence of the short-term immunity modification.

Regardless of pulsed or non-pulsed birth as well as the length of the $B(t)$ period ($B_{sd}$), the mean time point of the entry of naïve juveniles into the population ($B_{mean}$) was consistently lower (earlier) in scenarios including the 5th replacement of migrants modification. Without the 5th modification, $B_{mean}$ was selected to occur late leading to the contribution of naïve juveniles in the major autumn infection peak. In most scenarios, the ratio of migrants to residents ($Pr_M$) was highly skewed towards migrants: 3-4 times the resident population.

In general, across the first 15 scenarios that included the 5th replacement of migrants modification, the arrival peak of migrants ($A_{mean}$) occurred very early with respect to the pre-set window (early August). In contrast, without the 5th replacement of migrants modification, peak arrival dates occurred in mid-October. The shape of the arrival curve ($A_{sd}$) was consistently in the lower half of the pre-set parameter range and was particularly low, i.e. reflecting a quick and synchronised arrival, in the best fitting scenarios. Not much variation was found in the timing ($R_{mean}$) and shape ($R_{slope}$, $R_{kurt}$) of the replacement of migrants curve ($R(t)$). However, across all model scenarios including the 5th modification, the mean part ($R_{mean}$) was always at the upper boundary and therefore as early as possible. The amplitude of the replacement curve ($R_{amp}$) varied but was consistent within the upper half of the possible parameter range (0.35-0.6).

The sensitivity analysis revealed a correlation pattern between the optimized parameters. In the top ranked model scenario that included all except the 1st birth pulse modification, the immune rate ($\sigma$) and the ratio of migrants ($Pr_M$), as well as the immune rate and the amplitude of the replacement of migrants ($R_{amp}$), had the highest correlation coefficients (Figure 4.4). These correlations were also the most pronounced correlations across all 32 scenarios. Parameters defining the entry of naïve juveniles ($B_{mean}$, $B_{sd}$) and the arrival of migrants ($A_{mean}$, $A_{sd}$) were also often correlated.
Discussion

Mathematical modelling has great potential to probe the complex dynamics of infectious diseases and identify the mechanisms of transmission. Therewith, they may also indicate approaches for prevention and control that may help shape national and international public health policy (Heesterbeek et al. 2015). In this study we used a mathematical modelling approach to evaluate the main hypotheses that have been suggested to drive local AIV dynamics in wild bird populations influenced by bird migration. To evaluate our models, we fitted the predicted infection dynamics to a unique sampling dataset of a year-round, small-scale avian influenza virus surveillance study in the key European host species, the mallard (van Dijk et al. 2014a). We found that one particular mechanism, the local replacement of migrants during the peak migration period, contributed most significantly towards better model predictions. The importance of migratory replacement supports the general perception that animal migration plays a central role in wildlife disease dynamics by spreading pathogens across the globe (Altizer et al., 2011) and, as specifically supported by our study, by facilitating local AIV infection dynamics. In addition, however, our models provide strong indications that it is not only simply the occurrence of migrants but, most importantly, how migration takes place over time. Migratory populations and their individuals may migrate highly synchronised and visit stop-over sites all at once, or they may differ in timing leading to several waves of migrants and to extended periods during which migrants arrive at and depart from stop-over sites. In most species, including the mallard (Fransson and Petersson 2001, Bakken et al. 2003), the migratory season protracts over several weeks up to a few months. Mallards migrating south via The Netherlands in autumn, breed in the northern regions, ranging from Scandinavia, the Baltic and north–west Russia and overwinter in an area stretching from Denmark, to northern France and Britain (Scott and Rose 1996). Therefore, mallards visiting The Netherlands during autumn migration or remaining in The Netherlands to winter come from a vast geographic area leading to considerable variation in migratory timing. This is likely to lead to the mechanisms behind migratory replacement used as a modification in our model: arriving migrants stay in the area for a period of time after which they move on and are replaced by newly arriving individuals. As a consequence, individuals that have been exposed to AIVs prior to arrival or at the duck-decoy and are protected against re-infection by means of AIV antibodies, are replaced
by potentially susceptible individuals that may perpetuate or even invigorate local transmission dynamics.

Besides the strong effect of the replacement of migrants, models with better predicting power also included the modification differential susceptibility (higher transmission rates in migrants compared to residents). This is not surprising, since the observed data already showed that infectious migrants likely contributed significantly to the major AIV prevalence peak during autumn (van Dijk et al. 2014a). Combining the evidence from the model and the empirical study, we can conclude that migrants did not exclusively affect the resident population through their often suggested role in the introduction of new virus strains and the general increase in host-densities (Gaidet et al. 2012, Hill et al. 2012), but had an active part in the virus perpetuation and transmission within the duck-decoy. Verhagen et al. 2014 found a similar result at the same location, albeit focusing on the autumn AIV infection peak only, which was induced by a H3N8 AIV subtype. The vast and unknown geographical origin of the migrants visiting the duck decoy could again explain this pattern. Most migrants might have been naïve to the subtypes that some migrants may have introduced from locations most individuals never visited before. Since migration is a large-scale multi-species phenomenon, this might always be the case and it questions the hypothesis that migrants have an exclusive role in just introducing viruses and thereby affecting the resident populations only. Again, the question of how migration evolves in time and space and how synchronised individuals and populations are during their migratory journey (“Migratory connectivity”: see Chapter 5) is central in this discussion.

There are several non-mutually exclusive mechanisms that may explain why the better predicting models had an increased susceptibility in individual migrants. Empirical studies have shown, that the physiological challenges accompanied with the migratory journey and the apparent trade-offs with the immune system can reduce their immunocompentence and render migrants more susceptible (Buehler et al. 2008). It has also been suggested that migrants are generally more susceptible, since their immune system is less specialised but adapted to cope with the exposure of different and disparate environments and their pathogens (Waldenström et al. 2002).

Most northern hemisphere AIV surveillance studies in wild bird populations show similar patterns of pronounced late summer - early autumn infection peaks (e.g. Munster et al. 2007, Hénaux et al. 2013). Clearly, our model scenarios are ranked by their ability to capture this pronounced feature of the
entire annual infection dynamic. While differential susceptibility between migrants and residents seems to assist the predictions of the infection peak, the overall importance of this parameter reduces the informative power of the remaining evaluated mechanisms, like the birth pulse, the short-term immunity and the epidemiological state at which migrants enter a resident population. However, those mechanisms might still be crucial. Indeed, the correlation matrix (Figure 4.5) shows that the recovery rate ($\gamma$) and the immune rate ($\sigma$) could be parameters of importance and are highly correlated with parameters that significantly influence the autumn infection peak, like the ratio of migrants to residents ($Pr_{mig}$) and the amplitude of the migratory turnover ($R_{amp}$).

Birth pulses have also been shown to be of importance in infection dynamics in the wild (Hénaux et al. 2013), and appeared to be fundamental to produce annual infection peaks in empirically validated disease models (Hosseini et al. 2004, He 2005, Begon et al. 2009). Hénaux et al. (2013) in particular showed that the early autumn AIV infection peak in a major host species, the blue-winged teal ($Anas discors$), across the North American continent was mainly due to infections in naïve juveniles and that only a small proportion of adults were within the susceptible pool and contributed to the transmission dynamics. Although knowledge on the number of migrants was not included in this study, the results raise questions whether the underlying geography of the locations and the accompanied differences in the migratory strategy of birds could lead to other conclusions than found by Hénaux et al. (2013). Migration routes across the North American continent are grouped into four major north-south stretching flyways and phylogenetic analysis of AIV indeed indicate that those flyways represent corridors for gene flow with more restricted east-west gene flow, suggesting that migration of waterfowl occurs on a large scale from north to south with little longitudinal mixing (Lam et al. 2012). The preferentially north-south migration across a broad east-west front may restrict the origin and number of migrants within wetlands along the different migratory routes and reduce their influence on the local AIV infection dynamics. In contrast, the wetlands in central-northern Europe (e.g. The Netherlands) are within a bottleneck of the East-Atlantic Flyway in which most routes of waterfowl from a vast geographical origin merge (Scott and Pose 1996). However, at least one particular location in North America also seems to feature as a migratory bottleneck: Delaware Bay in the east-coast of North America. Every spring thousands of migratory shorebirds aggregate within the bay to feed on horseshoe crab eggs and thereby enhance AIV prevalence within migrants as
well as in the resident duck population by as much as 17 times compared to other locations (Krauss et al. 2010).

Our modelling approach provides new insights into the relative importance of demographic and epidemiological parameters that may predict AIV prevalence dynamics in wild birds. Despite the increasing efforts in the surveillance of wildlife diseases and the enormous increase in our understanding of e.g. virus diversity and evolution, temporal patterns of occurrence and host ranges (Olsen et al. 2006, Munster et al. 2007), understanding the ecological and immunological drivers of local as well as large-scale AIV infection dynamics remain a challenge. For our system, the models reveal the importance of migration and notably the timing and strategy of migration. However empirical data on the ratio of migrants to residents, the temporal patterns of arrival and most importantly the magnitude of turnover in the migratory population is lacking. Knowledge of these parameters would allow us to use mathematical models that go into more depth and estimate e.g. transmission-, recovery- and immune rates, the key processes in host-pathogen interactions (McCallum 2000). Therefore, we believe that besides unravelling the mechanistic understanding of infection dynamics, understanding demographic patterns are equally as important as virus detection. This notably holds for systems that host large numbers of migratory birds for parts of the annual cycle.

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Timing is crucial for consequences of migratory connectivity

Migratory connectivity can have important consequences for individuals, populations and communities. We argue that most consequences not only depend on *which* sites are used but importantly also on *when* these are used and suggest that the timing of migration is characterised by synchrony, phenology, and consistency. We illustrate the importance of these aspects of timing for shaping the consequences of migratory connectivity on individual fitness, population dynamics, gene flow and community dynamics using examples from throughout the animal kingdom.

Exemplarily for one specific process that is shaped by migratory connectivity and the timing of migration – the transmission of parasites and the dynamics of diseases – we underpin our arguments with a dynamic epidemiological network model of a migratory population. Here, we quantitatively demonstrate that variations in migration phenology and synchrony yield disease dynamics that significantly differ from a time-neglecting case. Extending the original definition of migratory connectivity into a spatio-temporal concept can importantly contribute to understanding the links migratory animals make across the globe and the consequences these may have both for the dynamics of their populations and the communities they visit throughout their journeys.
Introduction

Billions of animals migrate across the globe every year and it is widely acknowledged that the use of different sites has consequences for migrant fitness and the dynamics of their populations as well as for the communities visited (Webster and Marra 2005, Marra et al. 2010, Bauer and Hoye 2014). Migratory connectivity describes the “geographic linking of individuals and populations between one life cycle stage and another” (Webster et al. (2002), p. 76, Salomonsen 1955). It is a concept that can importantly contribute to answering a range of fundamental and applied questions and therefore, has been enthusiastically embraced by the scientific community. Although predominantly applied in studies of migratory birds, it is equally applicable to migratory animals of other taxa (e.g. Godley et al. 2010, Miller et al. 2012). The importance of migratory connectivity is generally acknowledged; yet, most studies describe the nature of connectivity but surprisingly few quantitatively link consequences to the degree of migratory connectivity.

If we wish to develop it towards an explanatory and predictive concept, we need to gain a better understanding of the mechanisms behind the consequences of migratory connectivity and this requires an explicit consideration of time. The consequences of migratory connectivity broadly include those on individual fitness and population dynamics, gene flow and genetic mixing, and community dynamics and ecosystem function. Although it is widely agreed upon that these consequences result from the use of specific sites, conditions on these sites also change over time and therefore, timing of migration will not only shape the magnitude of these consequences but also their nature.

We suggest that the timing of migration is characterised by three dimensions – synchrony, phenology, and consistency (which may also be coined as ‘variation’ around a ‘mean’ timing and their ‘autocorrelation’). Migration synchrony describes how wide-spread over time individuals of a population migrate (Figure 5.1). At one extreme, all individuals migrate at the same time - synchronously (Orell et al. 2007), while at the other, individuals migrate at different times - asynchronously. Specific examples of asynchronous migration include differential migration (Colbeck et al. 2013), where (age-, sex-, or family-)subgroups of a population migrate at different times. Migration phenology describes the timing of migratory steps - arrival, departure and staging times at sites - relative to the phenology of other relevant processes, e.g. temporal
availability of key-resources or presence and abundance of other species and populations (Fig. 1). At the two extremes, the migrants’ presence on a particular site fully coincides with, e.g. resource peaks (‘matched’) or is completely separated from the availability of resources (‘mismatched’). Finally, **consistency** describes how repeatable migration phenology and synchrony are over time - usually over several migrations. (Note that consistency also exists in the spatial sense, i.e. the degree to which migrants return to the same locations in successive migrations (Jorgensen et al. 2010).

In the following, we illustrate the importance of considering timing for the consequences of migratory connectivity using examples from throughout the animal kingdom, and primarily consider the roles of phenology and synchrony. For a specific example of these consequences – the transmission of pathogens and parasites – we develop a dynamic network model to quantitatively 

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**Figure 5.1:** The timing of migration – here exemplarily from a non-breeding site via an intermittent staging to a breeding site – can be characterised by synchrony (left panel) and phenology (right panel). Migration synchrony describes in how far individual migrants travel at the same time, i.e. synchronously, or at different times, i.e. asynchronously. Migration phenology relates the timing of migration to the phenology of resources or to that of other populations and species, with which migrants interact, e.g. via competition, predation, etc. The degree of coincidence between migrant visitation and resource availability (upper-right panel) determines the migrants’ fitness consequences, which under complete overlap can range from positive when resources are concerned to negative when it characterises the presence of predators.
demonstrate how changes in phenology and synchrony affect disease dynamics. We would like to emphasize that we do not aim at compiling a comprehensive review of the consequences of migratory connectivity here (for such, see e.g. Boulet and Norris 2006) but hope to stimulate discussion and increase awareness of the effects of variations in migration phenology and synchrony.

Consequences of migratory connectivity shaped by timing

Individual fitness and population dynamics

A variety of factors can affect a migrant’s fitness (Figure 5.2): Abiotic conditions, e.g. temperature, precipitation or wind, influence energy expenditure during residency (e.g. thermoregulation) and locomotion (e.g. flight); resource availability and abundance of competitors determine how fast migrants can replenish fuel reserves (Stahl et al. 2006, Wittwer et al. 2015); and predators pose mortality risks (Middleton et al. 2013) or spark a range of non-lethal effects (Morrissette et al. 2010). All of these factors change over time, usually seasonally but often at smaller time-scales, at time-scales similar and thus, relevant, to the visitation of migrants. Therefore, variations in the phenology of migration will lead to a population experiencing on average different resource levels, abundances of competitors and predators, (‘phenological match/mismatch’: Johansson et al. 2015), and migration synchrony determines the within-population variation with regard to the overall effects of these factors. If, for instance, resource availability changes as a consequence of natural decay or due to finite resources being exhausted, early migrants would benefit from abundant resources compared to late migrants in an asynchronously migrating population. This is exemplified in a population of Arctic breeding geese, where individuals that arrived at stop-over locations at the peak of vegetation growth had a higher breeding success (Kölzsch et al. 2015).

Similarly, within-population competition may be alleviated under asynchronous migration while it is fully effective under synchronous migration (Skoglund et al. 2011), e.g. as in the exclusion of competitively inferior individuals from high-quality foraging patches (Eichhorn et al. 2009, Beauchamp 2012). Alternatively, synchronous migration can be beneficial if the joint consumption of a resource increases its quality or productivity, as in the
case of grazing by migratory geese on a spring stop-over site (Stahl et al. 2006) or the increased productivity of the African savannah through the temporal grazing of migratory herbivores (Holdo et al. 2007).

Figure 5.2: The consequences of migratory connectivity can be manifold, ranging from consequences on individual fitness and population dynamics to those on community dynamics and ecosystem function. All of these consequences can be modified and shaped by the timing of migration - its phenology and synchrony, via a suite of different factors and mechanisms.

<table>
<thead>
<tr>
<th>Migration Synchrony</th>
<th>Migration Phenology</th>
</tr>
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<tbody>
<tr>
<td>... determines level of resources available for early and late migrants if resources change over visitation period.</td>
<td>... relative to phenology of resources determines match or mismatch between migrant requirements and resource availability.</td>
</tr>
<tr>
<td>... if conditions change over visitation period, early and late migrants experience different weather conditions, with potential fitness consequences.</td>
<td>... relative to seasonality of places and variability of weather can affect refuelling and survival.</td>
</tr>
<tr>
<td>... determines magnitude of density-dependent interactions.</td>
<td>... relative to phenology of other species sets the specific levels of competition, facilitation or other interactions.</td>
</tr>
<tr>
<td>... determines predation risk, hunting pressure or disturbance levels experienced by early/late migrants if these change over visitation period.</td>
<td>... relative to predator phenology or hunting schedules determines mortality risk experienced by migrants.</td>
</tr>
<tr>
<td>... affects pair formation and mating.</td>
<td>... relative to phenology of breeding places can result in variations in reproductive success and contribution to the following generation.</td>
</tr>
<tr>
<td>... may separate infected and uninfected individuals, changing transmission and prevalence.</td>
<td>... in relation to variations in susceptibility or environmental parasite pressure can alter prevalence levels over time.</td>
</tr>
<tr>
<td>... determines whether the input of nutrients or energy results in resource pulses, cascading through the local food web.</td>
<td>... relative to phenology of community determines the pathways through which inserted nutrients and energy enter local community.</td>
</tr>
<tr>
<td>... determines whether propagules are inserted simultaneously which enhances probability of establishment, e.g. via mass effect or numerical domination.</td>
<td>... sets the timing of nut relative to the phenology of community, which affects fate and effect of propagules, e.g. probability of establishment, pollination, etc.</td>
</tr>
<tr>
<td>... determines interaction strength with resident species and populations.</td>
<td>... determines which trophic processes in a local community are affected by migrants.</td>
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The level of predation (incl. hunting) may also change at the time-scale of migrant visitation, e.g. as resulting from seasonal hunting permissions or mobile predators. For instance, hunting on spring-migrating geese in Russia is permitted during 10 days of peak migration and individuals migrating outside this 10-day hunting window experience much lower mortality risks (Mooij et al. 1999). Similarly, late-migrating sandpipers responded to the arrival of predators (peregrine falcons, *Falco peregrinus*) on a common stop-over site with behavioural changes, e.g. increased vigilance, reduced foraging and consequently, reduced migration speed – behaviours that early-migrants failed to show (Hope et al. 2014).

**Gene flow and genetic mixing**

Migratory connectivity can influence the degree of gene flow between populations – as a result of either spatial or temporal segregation (Webster and Marra 2005, Bensch et al. 2009, Moussy et al. 2013). Although it is commonly acknowledged that strong (spatial) migratory connectivity can lead to limited or no gene flow, to local adaptations and ultimately, speciation (Bensch et al. 1999, Fraser and Bernatchez 2005), temporal segregation can have the same effects. A prominent example is the European blackcap (*Sylvia atricapilla*), in which there is no or very little gene flow between two sub-populations despite them meeting at a common breeding site. This is mainly explained by differences in arrival and onset of breeding between these sub-populations that segregated them temporally and resulted in assortative mating, restricted gene flow and ultimately, phenotypic divergence (Berthold et al. 1992, Bearhop et al. 2005).

**Community dynamics and ecosystem functions**

With their movements, migrants connect widely separated and diverse communities and ecosystems, influence their structure and dynamics through a variety of transport and trophic effects (Bauer and Hoye 2014). Clearly, migratory connectivity describes which communities and ecosystems are linked by migratory movements but the phenology and synchrony of visitation are also profoundly important to assessing the influence migrants can have on these communities. The timing of migration relative to resident phenology is fundamental to the strength and direction of migrant-resident interactions (Yang and Rudolf 2010) and can influence key-features of communities (Nakazawa and Doi 2012). For
instance, migrants can only be important pollinators if their visits coincide with peak flowering, e.g. Lesser long-nosed bats (*Leptonycteris yerbabuenae*) time migration to coincide with peak flowering in the cacti-populations along their way (Fleming 2004). Similarly, if parasite prevalence shows a marked seasonal dynamics, transmission may be restricted to sites where high prevalence and migrant visitation coincide (Hoye et al. 2011).

Effects of migrants on communities also can also be shaped by the synchrony of migration. For instance, the simultaneous input of nutrients may constitute resource pulses, which can profoundly alter demographic rates and abundances of interacting populations, with cascading effects that may persist long after the pulse is extinguished (Holt 2008). Also asynchrony in migration can have attendant consequences for communities and ecosystems, e.g. in partially migratory freshwater fish the proportion of the population migrating determines, via various intermediate steps, the transition between alternative stable states in a lake ecosystem (Brodersen et al. 2008).

**Transmission of parasites and disease dynamics**

A specific process that highly depends on migratory connectivity and can have implications on several organisational levels is the transmission of parasites and the dynamics of diseases. Infections impair the fitness of migrant hosts, e.g. directly through increased mortality but also indirectly through costly immune responses. Disease symptoms may range from fatigue, reduced foraging or movement (Adelman et al. 2010), which may knock-on to lower fuelling rates, later departure and eventually, in reduced reproductive success or survival. Depending on the proportion of a population being infected and the severity of effects, this may severely influence population demographic rates (Hudson et al. 2002b). On the community level, parasites can change the outcome of interactions such as competition and predation and thus, ultimately species coexistence and diversity (Holt and Dobson 2006).

If we want to understand the dynamics of parasites within migratory host populations, we need to consider migratory connectivity and the timing of movements (Altizer et al. 2011, Møller and Szep 2011): If individual migrants visit the same sites at the same times, they are thought to encounter the same variety of parasites and prevalence in the population is driven by local (re-)infections (Hudson et al. 2002b). In contrast, if migrants visit different sites or the same sites at different times, they potentially encounter a different diversity and abundance of parasites (Kamiya et al. 2014); once these
individuals congregate on a common site, they may harbour, and exchange, a greater variety of parasites (Gaidet et al. 2012).

Considering time explicitly is required for predicting the consequences of migratory connectivity to parasite prevalence and dynamics for several reasons: First, prevalence may vary over time, e.g. resulting from variations in environmental conditions (Reperant et al. 2010a), density of potential hosts (Gaidet et al. 2012) or the influx of immunologically naïve individuals, such that there are periods during which transmission is more likely than in others (Hoye et al. 2011). Secondly, infectious individuals need to actually meet susceptible (un-infected) individuals to transmit parasites, which might be efficiently prevented when infected and uninfected individuals migrate at different times. For instance in Monarch butterflies (Danaus plexippus), individuals infected with a protozoon parasite migrated at lower speeds than their healthy conspecifics (Bradley and Altizer 2005) and such “migratory escape” introduced a barrier to the spread of parasites that consequently reduced parasite-prevalence in the population (Altizer et al. 2011, Hall et al. 2014).

To underpin our verbal arguments, we used a simple model to demonstrate how the prevalence of pathogens in a population of migratory hosts may change when migration phenology and synchrony are varied. To this end, we combined a dynamic network model with an epidemiological and a migration model and followed the prevalence of pathogens in the migratory host population over time. The population (‘network’) consisted of individuals (‘nodes’), which may be linked via ‘edges’. Links between individuals form and dissolve probabilistically over time while the average number of links per individual in the network is preserved. Individuals were additionally characterised by their infection status and location – and these could change as a result of infection dynamics and migration, respectively.

*Infection dynamics*

We used a SIS (susceptible-infectious-susceptible) model (Keeling and Rohani 2008) with no immunity or latent phase. Susceptible individuals can be infected with probability, $\tau$, if connected to an infected individual and infected individuals recover with probability $\gamma$, and re-enter the pool of susceptibles. Although we had no particular pathogen or disease in mind, we used parameter values of $\tau = 0.2$ and $\gamma = 0.07$, i.e. infections lasted on average 14 days.

*Migration*

We considered a simple type of migration from a starting location to a destination, i.e. assuming strong migratory connectivity. Migration was
instantaneous and at, or around, a mean migration date, \( t_{mig} = 100 \). As we assumed the two locations to be distant, no links (and thus, no pathogen transmissions) were allowed between individuals at disparate locations.

**Scenarios**

We changed migration synchrony by varying the standard deviation around the mean migration date from \( \sigma = 0 \) to 10 and thus, from completely synchronous to highly asynchronous. Please note that the scenario on ‘complete synchrony’ is equivalent to aggregating over time. Secondly, we introduced a 20-day period of elevated (environmental) pathogen pressure (or increased susceptibility to infections) at one location and varied its onset relative to the timing of migration from day \( t = 60, 90, \) to 120, i.e. before, coinciding with, or after migration, respectively. During this period individuals at the starting location were additionally infected with a probability of 0.5.

**Results**

Both migration synchrony and phenology importantly shaped (local) disease dynamics (Figure 5.3). Changing synchrony changed the prevalence of pathogens (Figure 5.3a-b): In a completely synchronously migrating population, prevalence remained at the same level on both starting and destination location. However, if migration was spread out over time, prevalence gradually decreased at the starting location and gradually increased at the destination and was thus considerably lower than under complete synchrony.

Changing the timing of elevated pathogen pressure relative to migration altered pathogen dynamics substantially (Figure 5.3c-d). While pathogen pressure at the starting location obviously could not change prevalence if it was elevated after migration, it significantly increased prevalence when it was elevated before or during migration. The prevalence at departure from the starting location then spilled over to, and influenced, the prevalence at the destination location (Figure 5.3d).

Thus, although the underlying epidemiology remained unaltered, both migration synchrony and phenology importantly shaped (local) disease dynamics with the resulting prevalence varying widely and substantially differing from the time-aggregated case. Naturally, our modelling exercise is only a first step and comprehensive studies are required that apply the model to specific migrants, pathogens and parasites, extend it to accommodate more complex migrations of a variety of migrants, and include effects of parasites on host state, behaviour and demographic parameters. For more details on model description, scenarios and results and see Appendix A.5.1-3.
Conclusions

Migratory connectivity is an important concept for the links migrants make between different parts of the world; its implications are far-reaching and can be immense: the dynamics, conservation and management of migratory populations, the effects of potential habitat and climatic changes (Bauer et al. 2008), structure and dynamics of separated communities (Bauer and Hoye 2014), and the spread of parasites, including those with zoonotic potential.
In addition to the exclusive consideration of spatial links in its original definition, we have shown here that all potential consequences of migratory connectivity can depend on the timing of migration – its phenology and synchrony.

Migration phenology has long been acknowledged as vital for our understanding of the migrants’ population dynamics – numerous studies have shown its importance for individual fitness, population demographic rates as well as for the transmission of parasites and the interactions with resident communities. An individual’s phenology results from cues that trigger migration (Bauer et al. 2011, McNamara et al. 2011) and other life-history processes and is the result of adaptations to (local) conditions, to the variability in these conditions, and to interactions within populations or with other species (Reed et al. 2010). The level of synchrony in timing of migration then results from the variability between individuals in the use of and response to these cues and the conditions experienced (Harrison et al. 2011). Additionally, migration synchrony might be influenced by a variety of processes, e.g. variation in fueling rates (Seewagen et al. 2013), sex-specific constraints and selection pressures (Saino et al. 2010), or delayed departure of infected individuals (Hoye et al. 2012a), and it may vary for different migratory steps or between breeding and non-breeding migration. Furthermore, the level of migration synchrony will be generally higher in migrants travelling in groups, e.g. fish shoals, herds, swarms, as migration routes and timing result from group decisions (Conradt and List 2009) and with a cultural transmission of migration behaviour (Harrison et al. 2010).

One might argue that the original definition of migratory connectivity implicitly contains a time-dimension as migrants visit the various places at different times of the year. However, we think that time needs to be made explicit, often at a higher resolution than implicitly contained in the original definition, as we would otherwise neglect consequences that are directly shaped by phenology and synchrony of migration. [An analogy of the implicit-versus-explicit consideration of time could be drawn from network analyses: In most ecological applications to date, networks are considered as time-aggregated networks; however, the dynamics, resilience and stability of time-ordered networks can be fundamentally different from time-aggregated networks (Blonder et al. 2012).

As we have exemplarily shown, the dynamics of pathogen infections in migratory populations can substantially differ if the timing of migration is
considered explicitly – changing synchrony and phenology altered population prevalence over long periods although the underlying infection dynamics remained unchanged. Aggregating over time - as in the original definition of migratory connectivity - implicitly assumes that migrations are completely synchronous and disregards the relevance of other processes, which, however, are crucial for understanding its consequences.

Obviously, the relevance of timing may vary, e.g. between periods and places, but whether timing can be neglected or not depends on the consequences of migratory connectivity under consideration and their hypothesized mechanisms. Both consequences and their underlying mechanisms determine which spatial and temporal scales are required for their detection, and in turn, the spatial and temporal scales required have implications for the choice of empirical methods: Various tracking methods exists to date, all of which set very different yet strict limits to the spatial and temporal resolutions that can be achieved (Boulet and Norris 2006), and therefore, the choice of methodology determines, and possibly restricts, the consequences and mechanisms that can be identified.

This sets the basis for several exciting challenges for future research. A prime need is a sound theoretical basis for the consequences of migratory connectivity and the role of timing in shaping these. In particular, we need theoretical studies that develop predictions for specific consequences of migratory connectivity and explore their mechanisms (Taylor and Norris 2010), investigating the respective roles of phenology, synchrony and consistency in influencing the fitness of individuals, population dynamics, gene flow and community dynamics and ecosystem functioning. Network approaches might be particularly useful when consequences are modified by heterogeneous contact structures that change over time. For instance, several community and ecosystem consequences of migratory connectivity result from direct interactions (‘contacts’) between migrants and residents but the intensity and nature of these interactions critically depend on the numbers of migrants and their timing – phenology and synchrony (Figure 5.2): Whether parasites will be successfully introduced into a resident community, which pathways imported nutrients and energy take, or whether dispersed seeds establish in a resident community – depends on phenology and synchrony of migration and can be explored with dynamic network models.

A complimentary approach are behaviour-based migration models, which can explicitly take into account behavioural flexibility and constraints in responding
to climatic and habitat changes (Hedenström et al. 2007, Barta et al. 2008, Fagan et al. 2012), the variable number and importance of sites that constitute migration routes (Iwamura et al. 2013) but also fundamental differences in migration strategies, such as different modes of locomotion (Hein et al. 2012). Although for many species and populations we are still at the stage of identifying the places to which individuals migrate, we urge for an extension of the original, exclusively spatial definition of migratory connectivity into a spatiotemporal framework. Going beyond the descriptive stage of migratory connectivity requires us to be explicit about its consequences, their mechanisms and the spatial and temporal scales alike.

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Movement patterns of Sanderling (*Calidris alba*) along the East Asian Australasian Flyway and a comparison of methods to identify crucial areas for conservation

Most migratory shorebird populations around the world are in jeopardy, none more so than those of the East Asian Australasian Flyway (EAAF). In order to preserve these highly mobile species detailed understanding of their use of fuelling and resting sites along the flyway is required. In this study we used light-level geolocators and new analytical tools to reveal individual breeding locations and detailed migration routes of 13 Sanderlings (*Calidris alba*) that spend their non-breeding season in South Australia. We then used these individual migration routes to identify the timing and location of important stopping areas and compared this with assessments based on leg-flag resightings and count data. During both northward and southward migration Sanderlings were found to make extensive use of five major areas distributed along the Chinese coastline, the Yellow Sea and the northern end of the Sakhalin Peninsula. Insights gained from these individual migration routes highlighted inherent biases in only using count and resighting data to identify important fuelling and resting sites along the flyway. These findings suggest that individual movement data may therefore be crucial to effective conservation planning of shorebirds in the EAAF and elsewhere in the world.
Introduction

Animal migration is thought to have evolved in response to spatiotemporal variations in the abundance of resources and threats (e.g. Alerstam et al. 2003, Dingle and Drake 2007, McKinnon et al. 2010). Despite the immense costs involved in performing these regular and often long-distance journeys, migration is a common and widespread phenomenon in the animal kingdom (Dingle and Drake 2007). In particular, the majority of shorebird species are migratory (Kirby et al. 2008), some of whom perform migratory movements of up to 30,000km per year (e.g. Harrington 2001, Gill et al. 2009, Minton et al. 2010).

In order to accomplish the physiological demands of migration, most migratory birds partition their journey into a series of flights interrupted by periods of intense refuelling and resting (Piersma 1987, Colwell 2010). Consequently, most migrants depend on a network of suitable fuelling and resting sites along their flyway for successful migration and survival. This reliance on multiple sites is thought to render migrants highly susceptible to changes on a local and global scale (Piersma and Baker 2000, Runge et al. 2014) and, as a result, migratory populations across a wide range of taxa have declined in recent decades (Wilcove and Wikelski 2008). Migratory shorebirds across all global flyways are emblematic of these declines, especially on the East Asian-Australasian Flyway (EAAF) (Bamford et al. 2008, Amano et al. 2010).

Effective protection of highly mobile species is predicated on detailed understanding of how migrants make use of their flyway, and hence the areas and sites that are crucial for conservation (Holdo and Roach 2013, Runge et al. 2014). Bird counts and bird banding programs, including leg-flag resightings, have traditionally been used to develop an initial understanding of migratory movements and to initiate conservation measures (e.g. Bamford et al. 2008, Minton et al. 2011). These methods are highly dependent on the spatiotemporal distribution of observers. Accurate tracks from individual migratory shorebirds equipped with satellite transmitters have revealed considerably more detail on migration schedules and routes (Gill et al. 2009, Battley et al. 2012). Despite the ongoing development of lighter devices, the use of satellite transmitters is restricted to a limited number of species of larger body mass (Bridge et al. 2011). Light-level geolocators (hereafter geolocators) are considerably lighter (<1g), and therefore provide the opportunity to track smaller migrants greatly expanding the number of species that may be studied. Initial application of geolocators to shorebirds has provided valuable
information on the migratory movements of a number of species using the EAAF (e.g. Minton et al. 2010, Minton et al. 2013). However, our ability to make detailed and accurate descriptions of migratory movements based on geolocator data has, so far, been hindered by coarse spatial resolution (Lisovski et al. 2012) and the inability to determine spatial location during the equinox – due to equal day length across the globe - as well as the breeding season due to constant daylight on the shorebirds’ Arctic breeding grounds. Consequently, the identification of critical sites used by large proportions of a population has thus far remained a key challenge to conservation along the EAAF. Yet, the ongoing development of geolocator devices and processing tools offers significant potential for progress.

In this study, we used new-generation geolocators recording the full light range, in combination with recently developed analytical tools, to determine individual movement tracks and associated uncertainty estimates, providing detailed understanding of Sanderling (Calidris alba) migration patterns along the EAAF. Sanderlings have a worldwide distribution and perform some of the longest distance migrations yet recorded (Lanting 1984, Reneerkens et al. 2009, Minton et al. 2013). The species breeds in Arctic locations along the north coast of European and Siberian Russia, in parts of Alaska, the Canadian Arctic and northern Greenland. During the non-breeding season Sanderlings utilise a wide range of overwintering habitats, including areas around 40-45°N in western Europe, North America, and Asia (MacWhirten et al. 2002), and down to the southern tips of South America, Africa, and Australia. Our study aims were twofold: (1) to provide a detailed description of the bi-annual migrations of Sanderlings along the EAAF and (2) to compare three different methods - (i) count data, (ii) leg-flag resightings, (iii) individual geolocator tracks - for identifying critical areas for shorebird conservation along the EAAF. Geolocator tracks were furthermore processed and compared using the most frequently used and hereafter referred to as \textit{simple threshold method} (Lisovski et al. 2012a) and a recently developed more sophisticated Bayesian framework, hereafter referred to as \textit{MCMC path estimates}. In describing the movements of Sanderlings along the EAAF, we further aimed to show that the recent advances in light-level geolocation allow us to (i) reveal detailed migration schedules and routes, (ii) estimate the location of the high Arctic breeding sites and (iii) describe and quantify the degree of within-population migratory connectivity over the annual cycle.
Methods

Animal capture and tracking data

A total of 44 Geolocators (Migrate Technology Intigeo-W65) were deployed in March 2012 at Canunda National Park, in the South East of South Australia (140°11'E, 37°37'S) under approval from the South Australian Department of Environment, Water and Natural Resources. Canunda National Park is an important overwintering site – used during the northern hemispheric winter - for Sanderlings, with typically between 200 and 400 individuals from October to April each year (Bamford et al. 2008). Sanderlings were caught, using cannon nets, in a single catch on the ocean beach where they forage and roost. Each bird received a leg-flag on their left tibia, on which a geolocator was fastened using Kevlar thread reinforced with Araldite resin cement. Geolocators weighed 0.65 g, making the total weight, including the flag, approximately 1 g. This represents < 2% of the Sanderlings’ mean (lean) body mass (see Appendix A.6.1. for morphological measurements). Based on multiple reports it appears that shorebirds readily adapt to carrying a geolocators on their leg and that the device has no significant effect on annual survival (e.g. Conklin et al. 2010, Niles et al. 2010). A total of 14 geolocators were retrieved (32%), 13 from birds caught over three successive days in November - December 2012 at the same location as they were deployed, and one from a bird shot in Sakhalin, eastern Siberia, in May 2013. Geolocator retrieval rates in other shorebird studies, conducted by the Victorian Wader Study Group and the Australasian Wader Studies Group varied between 10% (Great Knot) and 50% (Ruddy Turnstone). These retrieval rates reflect the ability to detect and catch the individuals as well as their site fidelity and not their actual survival probability. Other individuals carrying geolocators have been seen occasionally at this location and in the vicinity, including one as recently as September 2016. The bird shot in Siberia during northward migration in 2013 also indicates that detection rate is not 100% since the geolocator data revealed that this individual spent the non-breeding season 2012 in the proximity of the deployment site. All individuals, except the bird shot in Siberia (B013), were sexed molecularly, using the primers P8 and P2 according to the method described by Griffiths et al. (1998). We used a principal component analysis based on morphological measurements to assign B013 the status of female with a probability of 0.7 (see Appendix A.6.1 for details).
**Geolocator analysis**

Light intensity recordings from geolocators were used to firstly estimate the breeding sites of each individual and subsequently, using the derived breeding site position, to estimate the full migration path. **Breeding sites:** Sanderlings breed at latitudes above the Arctic Circle (Lappo et al. 2012a)(Lappo et al. 2012) and thus experience constant daylight during this part of their annual cycle. Conventional methods to estimate positions from light intensity recordings over time (i.e. geolocation by light) generally fail to produce reliable position estimates under 24hr daylight conditions as the light sensor generally does not record any variation in light intensity across the day (Lisovski et al. 2012). However, the light sensor in the geolocators used in this study recorded the full range of light intensities for each day, allowing the breeding location to be. We developed a template fit analysis to estimate the positions of the breeding sites. Separately for each individual light intensity records from the deployment site in Australia, recorded on the bird during the stationary period after deployment or before retrieval of the device, were used to generate a calibration curve of light intensity as a function of zenith angles, using astronomical functions within the R package ‘SGAT’ (Wotherspoon et al. 2013). This calibration curve allowed generation of expected light at any location for a given time/zenith angle. Using the individual, geolocator-specific calibration curves, predictions were made on the temporal variations in daily light intensity for the entire breeding season for every 50x50km grid cell across the entire Russian Arctic. Next, the predicted light values were compared with the observed light data. We calculated the percentage of single light intensity recordings that exceeded the predicted values within each grid cell. Observations can only have been recorded within grid cells where 100% of the observed light intensity values were below the predicted values. This approach assumes that birds were resident during the entire breeding period. To correct for potential travel after arrival to and prior to departure from the Arctic we excluded observations from three days after entry and three days prior to departure from the region experiencing 24 hours daylight. In the best case scenario, when light intensity measurements are only little influenced by shading of any kind, the 100% likelihood contour line plotted across the potential breeding area is characterised by a v-shape (or u-shape) with the highest likelihood slightly above the minima of this contour line. We selected the closest position on land to this lowest latitudinal position above the 100% contour line. From data recorded on the deployment/retrieval site in South
Australia we estimated geolocation error to be <200 km. However, since the variation in sun elevation angle at the breeding grounds is low and signal to noise ratio is high (due to incubation and habitat) we expect location accuracy of the estimated breeding sites are supposed to be up 100-300km lower (for more details, code and explanations go to: https://github.com/slisovski/Geolocator-ArcticWader-BreedingSiteEstimation).

**Migration pathway**

Daily positions, and hence migration pathways, for each individual were estimated from raw light-level data using the threshold method of estimating positions based on sunrise and sunset events (Lisovski et al. 2012). Daily sunrise and sunset times as well as initial positions based on the simple threshold method were calculated using the R package ‘GeoLight’ (Lisovski and Hahn 2012). A light intensity threshold of 0.8 was used for all individuals. The corresponding zenith angle was defined from sunrise and sunset times recorded while the birds were at the deployment site. The defined zenith angle varied between individuals and ranged from 93.4 to 96.5. To derive more accurate positions we used a Bayesian framework that incorporates the observed sunrise and sunset times together with prior knowledge of Sanderling behaviour to provide location estimates with associated measurements of uncertainty. The used R package ‘SGAT’ (Sumner et al. 2009, Wotherspoon et al. 2013) uses Markov Chain Monte Carlo (MCMC) simulations that permit a spatial probability mask, prior definition of the error distribution of twilight events (twilight model) and plausible flight speed values (behavioural model), which collectively allowed us to refine the tracks derived from the sunrise and sunset times (detailed description of model assumptions: Sumner et al. 2009). The spatial probability mask is based on the premise, that during migration, Sanderlings are most commonly found on coastal sandy beaches, although they may also occur on tidal mud flats and the shores of lakes and rivers. Estimated positions were therefore considered to be more likely if close to a shoreline and independent of the habitat type: the relative probability assumed to decrease exponentially (from 4 to 1) with increasing distance from the shoreline \[1 + 5 \times \exp(-d/50000^3)\]. We used a spatial shoreline dataset with a 1:75 000 scale (http://shoreline.noaa.gov). For the twilight model the discrepancy between observed and expected times of twilight was assumed to follow a log-normal distribution. For sunrise positive values correspond to an observed sunrise occurring after the expected time of sunrise, whereas positive values for sunset
correspond to an observed sunset occurring before the expected time of sunset. We chose a conservative prior (log-normal distribution: meanlog = 1.65, sdlog = 0.9) since error in twilight detection is potentially very variable over the annual-cycle. For the *behavioural model* we assume that migratory shorebirds perform stepwise migrations, with relatively long staging periods in between periods of movement (Piersma 1987, Warnock 2010). We modelled flight speed (ground speed) using a gamma distribution (shape = 0.7, scale = 0.05) assuming that the speed with the highest probability was below one (i.e. the bird is most likely to be stationary at any given time), and that maximum flight speeds up to 80 km/h were likely to occur during migration (Pennycuick *et al.* 2013). For each individual we used these parameters and started by drawing an initial 10,000 samples for burn-in and tuning of the proposal distribution. One sample reflects one set of positions, between each twilight event, along the migration path. The proposal distribution is the conditional probability – here the spatial probability distribution of the individual - that is calculated after all available information was taken into account. A further 40,000 samples were drawn to visually evaluate chain convergence. A final draw of 5,000 samples was then generated to describe the posterior distribution. R code for location estimation analysis for each individual is available from the authors upon request.

*Analysis of migratory movements*

We used the median of the posterior distribution as our estimate for the most likely daily position of each individual, and hence their most likely migration path. We evaluated the timing of migration and whether the individual was moving on a given day during migration using a “first-passage-time” analysis: the fpt describes the time required for an individual to cross a circle with a radius of 500km (Fauchald and Tveraa 2003). In a second step, ftp was used to identify periods of residency – periods of stable ftp within the defined radius. All posterior distributions, 5,000 positions per day per individual, were put together to calculate the aggregated time the tracked population spent in each grid. Furthermore, those posterior distributions were used to analyse migratory connectivity and, in particular, the spatial spread of the individuals from the tracked population over time resulting from the temporal synchrony within the population (Bauer *et al.* 2015). To quantify the within population connectivity, a minimum convex hull was generated around the 0.6 and 0.95 quartile contour lines – the space that includes 60% and 95% of the samples forming the posterior distribution - across five-day intervals using the *mcp* function of the R
package adehabitatHR (Calenge 2006). The area where the convex hulls and the flyway, defined as the 0.4 quartile contour line of all posterior distributions from all individuals, overlap were used to quantify the spatial spread of the population for each five-day period.

Leg-flag resightings
To compare leg-flag resightings with our tracking results we used resightings of Sanderlings originally flagged on the coast of the south east of South Australia, where the geolocator devices were deployed (orange over yellow flags, total flagged = 3638). Leg-flag resightings from across the flyway have been drawn from the Australasian Wader Studies Group database (http://www.awsg.org.au/flagging.php). We further limited our use of resightings to those reported during the migration periods (northward migration: 15 April – 1 July; southward migration: 1 July – 1 November). Flag sightings were aggregated on a 500x500 km spatial grid.

Bird counts
Sanderling counts were extracted from Bamford et al. (2008; Page 91-93). This report estimated the size of shorebird populations within the EAAF based on a review of count data. Bamford et al. (2008) provide spatial information on counts per species and for the periods of northward migration, southward migration, breeding and non-breeding, recorded between 1979 and 2003. Here we used the count data from identified internationally important sites that regularly support 1% of the individuals of a population of one species or a subspecies (Criterion 6 of the Ramsar Convention). Maximum counts were aggregated on a 500x500 km spatial grid.

Results

Breeding sites
Estimated breeding site positions spanned an area between the New Siberian Islands of eastern Siberia, 300 km south to the mainland, and 1,300 km west to the Taymyr Peninsula (Figure 6.1). The highest aggregation of estimated breeding sites occurred on the New Siberian Island complex.
Migration pathways
Thirteen individuals were seen to perform a complete migration cycle from the deployment site in South Australia to the Arctic breeding grounds and back. Only one individual, ID2030, did not perform a complete migration. Instead, this individual departed on the 15th of May, made an extended stopover in Borneo (35 days), and another in the area of Hainan Island, southern China (35 days), before returning to Australia. We excluded this individual from all further analyses.

Northward migration: After deployment with geolocators, the Sanderlings remained at Canunda NP and departing for their first migratory leg between the 26th of April and the 10th of May 2012 (mean ± sd; Population: 2nd May ± 4.5
d; Females: 3rd May ± 5 d; Males: 1st May ± 4 d; Fig. 6.2b). In general, individuals performed a single long-distance flight from South Australia, across the equator, to the coasts of Vietnam and China. The coasts of Hainan Island, central China, Taiwan and the Yellow Sea were intensively used for extended stopovers (Figure 6.3a). All but one individual (ID2007) also made an additional stopover along the coast of the Sea of Okhotsk, around the northern end of Sakhalin Island. Sanderlings arrived on their breeding sites between 5th and 16th June 2012 (Population: 11th June ± 6d; Females: 11th June ± 5d; Males: 10th June ± 6d; Figure 6.2b). Northward migration, on average, was completed within 40 days, with a maximum of 48 (ID2003) and minimum of 35 (ID2007).

**Southward migration:** All tracked individuals departed their breeding sites between 13th July and 22nd August 2012 (mean ± sd; Population: 23rd July ± 11 d; Females: 1st Aug ± 19 d; Males: 19th July ± 6 d; Fig. 6.2c) after staying between 32 (ID2009) and 66 (ID2018) days on their breeding areas. Similar to northward migration, all but one individual (ID2006) used the coast of the Sea of Okhotsk as the first major stopover site after crossing the Arctic Circle (Figure 6.3); ID2006 instead used an inland route via Mongolia before stopping on the coast of central China and Taiwan. The majority of individuals had subsequent stops along the coasts of China, Taiwan and Korea, although considerably fewer individuals visited the Yellow Sea during southward compared to northward migration. In contrast to northward migration, all individuals made at least one additional stopover in tropical or sub-tropical regions (Philippines, Indonesia and Malaysia) before returning to Australia. Furthermore, more than half of the individuals (7) used at least one stopover site on the Australian continent before returning to Canunda NP and surrounds. Sanderlings arrived at these sites between 20th September and 12th November 2012 (Population: 9th October ± 27d; Females: 8th October ± 28d; Males 9th October ± 17d; Figure 6.2c). Southward migration, on average, was completed within 78 days, with a maximum of 108 (ID2038) and minimum of 57 (ID2019) days.

**Lag-fleg resightings**
To date 488 resightings of the 3638 individual Sanderlings banded in the southeast of South Australia have been reported during the migratory period. Of these, 208 were recorded during northward migration and 280 during southward migration. On northward migration the vast majority of resightings have been recorded in the Yellow Sea region (n=137; 66%). A further 26
resightings (12%) were recorded from the northern part of Sakhalin Island, Russia, and 25 (12%) from the coast of central China and Taiwan. During southward migration only one resighting was recorded within the Yellow Sea. The highest density of re-sightings during southward migration were instead reported from Japan (n=145; 55%), along with resightings from the northern end of Sakhalin Island, Russia, (n=31; 11%) and the coasts of central China and Taiwan (n=32; 11%).

**Count data**

After collating the maximum counts of Sanderlings from all *internationally important sites* on a 500x500 km grid, we retained a total of 12 such grid cells; 7 during the time period of northward migration and 11 during the time period of southward migration. Two areas adjacent to the geolocator deployment site in south-east Australia, two areas within the Yellow Sea (Yenchek National Nature Reserve and Linghekou, China) and three areas in Japan (multiple sites in central and south Japan) and southern South Korea (Nakdong Estuary) were identified for northward migration based on counts. For southward migration five additional areas of importance were identified: the northern end of Sakhalin Island (Sakhalinsky Bay, Russia), the south east of the Yellow Sea (Kum Estuary, South Korea), two in north-western Australia (Roebuck Bay and Eighty Mile Beach) and one in Tasmania (Blanchet Point).

**Comparison of methods for identifying important sites**

Based on the merged posterior distributions of all individual MCMC path estimate, five areas could be identified as being used extensively by the tracked individuals and are therefore classified as areas of major importance. The number of days spent within these areas based on the *MCMC path estimates*, *simple threshold estimates*, leg-flag resightings, and the sum of maximum Sanderling counts are shown in the tables in Figure 6.3. While the relative number of days spent between the important areas are concordant between the two different geolocator analysis methods, the leg-flag resightings and the bird counts overestimated the population’s use of certain areas (e.g. south and central Japan), and underestimated or even omitted the population’s use of other areas (e.g. the coastlines of central China and Taiwan; Figure 6.3).
Figure 6.3: Comparison of methods for identifying important stopover areas during Sanderling migration. Days spent at each area during northward (upper panel) and southward migration (lower panel) are tabulated for two analyses of 13 individual birds tracked using light-level geolocators, as well as the sum of maximum counts and leg-flag resightings. Percentage values indicate the relative proportion of time spent, all resightings, or all counts across all areas. Maps represent time spent for geolocation methods on a 100x100km grid, and the sum of leg-flag resightings or maximum counts on a 500x500km grid.
**Spatiotemporal patterns of migratory connectivity**

Before departure on northward migration, the spatial spread across the population of tracked individuals was low (60% convex hull: 1.5^{11} – 2.5^{11} km²; 90% convex hull: 1.3^{12} – 1.9^{12} km²) and hence the connectivity within the population was high. With the onset of northward migration spatial spread increased considerably, reaching a maximum of 1.4^{13}-1.9^{13} km² (60% and 90% convex hulls) between 3rd and 8th of May (Figure 6.4a). The area subsequently decreased, to 1.6^{12}-7.8^{12} km², between 23rd and 28th May (Figure 6.4b), before increasing again to 5.8^{12}-1^{13} km² between 2nd and 7th June, shortly before the Sanderlings’ arrived on the breeding grounds (where the population spread over 5^{11} - 1.9^{12} km²). Soon after the onset of southward migration, the area used by the Sanderlings increased, peaking at 1.6^{13}-2.3^{13} km² between 25th and 30th September (Figure 4b). Thereafter, the area used steadily decreased until the population returned to the relatively small area around the deployment site in South Australia.

**Figure 6.4:** Migratory connectivity within the Sanderling population over time. The spatial spread of the population for a given time period was calculated as the area of the minimum convex hull enclosing 60% (grey bars/dark grey polygon) or 95% (line bars/light grey polygon) of the combined MCMC-estimated paths from all 13 individual. The maps show two extremes - periods of the greatest (left) and least (right) connectivity within the population. The dashed line indicates the boundaries of the flyway used by the tracked Sanderlings.
Discussion

Comparison of methods for identifying important sites

When identifying areas of importance for the conservation of highly mobile species, several methods are frequently used. Our results from Sanderlings suggest that although individual tracking methods, leg-flag resightings and bird counts show considerable overlap, critical detail may be lost when relying on the latter two methods alone. All four methods assessed here invariably identified the coastline of the Yellow Sea as the major stop-over area for Sanderlings during northward migration, and both geolocator data and leg flag resightings indicated extensive use of a large swath of the central Asian coastline. All methods also highlighted the use of the West Australian coastline as Sanderlings returned to their overwintering site, and that these areas were skipped during northward migration. Indeed, our results suggest that areas of importance to the population may by underestimated or even missed when assessments are based solely on on-the-ground observations. While several clear areas of importance coincided between both geolocator analysis methods, the population counts and leg-flag resighting methods showed substantial differences. Critically, the importance of five areas, notably those in tropical and sub-tropical regions, was generally underestimated using leg-flag resightings, and even omitted when relying on count data. Strikingly, the coasts of central and southern Japan were prominent in the count data for both migration legs, and for southward migration in the leg-flag resightings, yet only one of the tracked Sanderlings (B013) showed a migration route via Japan.

Clearly, all methods discussed here have limitations and some of the discrepancies between the count data and the other three methods may partially be explained by the fact that we obtained tracking data from a small number of individuals from a single overwintering population only. Additional tracking data would undoubtedly improve the accuracy of our estimates, however, leg-flag resightings from Sanderlings caught and flagged at other overwintering sites along the flyway show very similar spatial patterns to the individuals flagged in South Australia (Minton et al. 2011). Finally, the count data presumably include individuals from several overwintering populations, and yet key sites used by our tracked individuals were not recorded in this data. Those sites might have not been counted, or do not appear in the Bamford et al. 2008 report due to other reasons, for instance the counts might have been too small to meet the 1% of the flyway population criterion. We also recognise that populations counts and leg-flag resighting studies are primarily intended
for purposes other than identifying crucial areas for conservation, including the study of population dynamics and estimating mortality rates. However, to date these data resources constitute the most extensive information on species-specific movements and are therefore frequently used to identify areas of importance and inform conservation planning. The discrepancy between counts, leg flag resightings, and individual movement data shown here highlights the value of integrating multiple data sources in order to inform conservation planning, prioritisation, and resource allocation on ground.

Geolocator analysis

The correspondence between both geolocator methods is initially surprising, given the low precision and accuracy of the simple threshold method (Lisovski et al. 2012). However, most shorebird species have a preference for open habitats, resulting in little noise in light intensity recordings. Moreover, the equinoxes – periods with comparatively low accuracy and precision in the simple threshold estimates (Lisovski et al. 2012) – did not coincide with periods in which the Sanderlings visited the five important fuelling or resting areas. The two methods might thus yield very different results in other species, especially in those using more vegetated or heterogeneous habitats. Additionally the MCMC path estimates feature important advantages over the simple threshold estimates in that the Bayesian method provides a framework that enables additional information, such as species-specific habitat preferences, movement behaviours and home range, to be incorporated. This greatly reduces the probability of erroneous, incompatible location estimates. Moreover, the method allows estimation of confidence intervals for location estimates that reflect the quality of the data. Finally, the method permits the estimation of a continuous path, whereby each observation is used and evaluated in relation to all other observations, rather than the relatively arbitrary qualification of each position in isolation and hence the potential omission or inclusion of positions derived by simple threshold estimates (Sumner et al. 2009).

Our template fit analysis allowed for the breeding areas of the tracked Sanderlings to be identified (Figure 6.1). Lending credit to the analysis, the 100% and 99% likelihood contour lines for the individual breeding areas showed identifiable and concurrent minima on or close to locations on land. Six individuals migrated and probably bred on the New Siberian Islands, well known as an area of breeding shorebirds including Sanderlings (Lappo et al. 2012). The breeding locations of two individuals were estimated just below the
New Siberian Islands in the coastal regions of the mainland. While Sanderlings are not recorded as breeding in this area (Lappo et al. 2012), it should be noted that based on our template fit analysis, locations on the Islands are equally likely and are well within the latitudinal accuracy of the position estimates. The location estimates for the remaining five individuals were west of the New Siberian Islands, as far as the eastern part of Taymir Peninsula (Figure 6.2). Both the eastern coastal areas of Taymir Peninsula and the Lena Delta are confirmed breeding areas of Sanderlings (Lappo et al. 2012).

The Sanderlings rapid and highly-synchronised northward migration (Figure 6.3) was in accordance with other observations on migratory birds (reviewed by Nilsson et al. 2013). In fact the tracked population spent twice as long on southward migration as northward migration. Such patterns are thought to be related to high selection pressure on a timely arrival at the breeding grounds (McNamara et al. 1998, Kokko 1999, Both and Visser 2001) with northward migration therefore considered a period under considerable time constraint. Similarly, the migratory connectivity within the population varied over time and between the two migration legs. As a result of the highly-synchronised northward migration, the area used by the population shrank by an order of magnitude once all individuals arrived on the central Asian coastline (Figure 6.4b). In contrast, spatial connectivity was very low during southward migration, to the extent that as some Sanderlings arrived on the overwintering grounds in South Australia others had only just made it to the Yellow Sea, lagging approx. 9,000km behind (Figure 6.4c).

We observed no differences between males and females in their timing during northward migration. However, the schedule of departure from the breeding grounds seemed to be sex-specific. All males departed within a relatively short time frame (14 days of one another), whereas all but one female departed both later, after the last male, and with considerably more inter-individual variation (Figure 6.2c). Although very little is known about sex-specific parental investment in Sanderlings, Tomkovich and Soloviev (2001) and Reneerkens et al. (2014) made the observation that only single birds cared for hatchlings and that more males attended broods from earlier clutches, while females predominantly cared for late clutches. This could explain our observed differences in departure dates between the sexes and suggests that some females may have had double-clutches, as observed in other areas (Reneerkens et al. 2009), with females caring for the second clutch.
During northward migration Sanderlings performed an initial long migratory ‘jump’ across the equator, after which they all used three to five stopping sites before crossing the Arctic circle and arriving at their breeding grounds. In contrast, the majority of Sanderlings used as many as six to seven stops during southward migration. This ‘hopping’ during southward migration and the latter stage of northward migration is similar to what has been found in Ruddy Turnstone (Minton et al. 2010) but contrasts with what we know from tracking studies of other species along the EAAF (Battley et al. 2012; Minton et al. 2013; Minton et al. 2010). These other species - Bar-tailed Godwit, Greater Sand Plover and Eastern Curlew - use one to two major stopover sites during both their northward and southward migrations. Sanderlings and Ruddy Turnstones are generalist feeders (del Hoyo et al. 2010) and may thus be less restricted in their habitat use than the other species. Alternatively, the relatively high number of stops observed in Sanderlings and Ruddy Turnstones may be related to body size (cf. Piersma 1987; Warnock 2010), both species being amongst the smallest species tracked within this flyway thus far.

Conclusions
Given the apparent value of integrating tracking studies with existing leg-flag resightings and count data to identify crucial areas for conservation, we urgently require more detailed individual migration tracks from the entire range of migratory shorebird species and populations of the EAAF. Study designs should emphasise acquisition of sufficient individual tracks to infer within and between population level distributions throughout migration. Increased understanding of how species and populations use the network of sites along the flyway would also assist predictions of how shorebirds are likely to respond to the rapid changes to their habitats along the flyway. Tracking studies, along with the systematic monitoring of the population through marking, resighting and counting, therefore form an essential part of the empirical research fundamental to conserving the many threatened migratory populations in the EAAF and elsewhere in the world.
Acknowledgements

We would like to thank the late Ren de Garais who (together with IS) first drew our attention to the presence of Sanderling carrying leg flags on the shores of the south east of South Australia. We also thank all members of the Victorian Wader Study Group (VWSG) for deploying and retrieving geolocators from Sanderlings. We specifically thank Roger Standen for providing the leg-flag resightings of Sanderlings, and Simon Wotherspoon and Michael Sumner for their work on the SGAT software and their support in the analyses. Heiko Schmaljohann, Theunis Piersma and one anonymous reviewer provided valuable comments on a former draft of the manuscript. Several individuals and organizations, notably the Norman Wettenhall Foundation, provided funding to the VWSG to conduct this geolocator project. Friends of Shorebirds South East also raised significant funding to pay for geolocators. Banding permits were supplied by the Australian Bird and Bat Banding Scheme and ethics/state banding approvals were provided by the South Australian authorities.
Synthesis: Lessons learned and questions unanswered

Half a century ago, people believed they had won the fight against infectious diseases, with improved conditions in hygiene, nutrition, drugs and vaccines leading to a steady decline in overall mortality (WHO 2000). The emergence of serious infectious diseases in recent decades, however, has made it clear that disease threats will persist and will remain one of the biggest challenges confronting humans into the future (WHO 2013). Most new infections enter the human population via infected wildlife or livestock, whereby pathogens infecting particular animal species undergo genetic modifications rendering them infectious to humans (Jones et al. 2008). As a result of population growth, increased urbanisation, land changes, greater travel and livestock production to meet the world’s expanding population, the likelihood of humans encountering zoonotic diseases is predicted to increase in the coming decades (Daszak et al. 2000, Jones et al. 2008, Keesing et al. 2010). In fact, 70% of the emerging infectious diseases in humans are zoonotic, with prominent examples including the severe acute respiratory syndrome (SARS) coronavirus, Hendra virus and HIV/AIDS. This has placed added urgency for gaining a more detailed understanding of the ecology of disease dynamics in wild animal populations. As a consequence, considerable amounts of resources have been allocated to virologists, epidemiologists and ecologists and this has significantly expanded our understanding of how pathogens affect individuals, populations, and even ecosystems (Tompkins et al. 2011). Despite these gains, however, these studies have also revealed there is still much to learn regarding the ecological and evolutionary dynamics of pathogens and how these influence a wide range of often interconnected temporal, organizational, and spatial scales. Thus, while
we know that interactions between pathogens and their hosts can have effects on the ecology of individuals, their populations, and even on entire ecosystems. We are still far from identifying the driving forces responsible for the dynamics of most wildlife infectious diseases, which hampers our ability to predict outbreaks and develop mitigating management plans. As an ecologist, I am fascinated by the complex interactions of host-pathogen systems and how these have the potential to affect the health of humans and animals. This has motivated me to accept the challenge to address some of the critically important questions concerning the ecology of wildlife infectious diseases.

Influenza A viruses (AIV) are arguably one of the most ubiquitous pathogens that infect animals and humans (Stallknecht and Brown 2008). Although the virus has been isolated from a wide range of vertebrate taxa, wild birds, particularly waterbirds of the order Anseriformes and Charadriiformes, are believed to be the natural reservoir of almost all AIVs (Webster et al. 1992). While the regular, low pathogenic avian influenza virus (LPAIV) cause only mild signs of disease if any (Kuiken 2013), some subtypes of LPAIV can evolve into a high pathogenic phenotype (HPAIV also known as the “fowl plague”), which can cause mortalities of up to 100% of the infected population (Alexander 2007). HPAIV have primarily been detected in domesticated ducks and poultry, with limited numbers of these viruses isolated from wild birds (Alexander 2007). However, the obvious potential for free-living birds to transmit this particular virus and spread it to both domestic animals and humans have made AIV one of the best studied wildlife diseases to date (Hoye et al. 2010). Despite these efforts, we still lack a clear understanding of two fundamental aspects of AIV transmission: 1) what are the drivers that contribute to the prominent seasonal infection patterns and 2) what roles do migrating birds play in affecting global and local infection dynamics. Those two major questions are the core of the research presented in this thesis.

In examining the literature of seasonal effects on animals, it became obvious that most ecologists use proxies, such as latitude, to estimate the seasonality of particular habitats. Surprisingly, studies addressing seasonality from the perspective of its effects on bioproductivity and how its seasonal variability that vary across the globe may affect other ecological processes such as host-pathogen interactions are largely absent. For this reason, I looked into ways of quantifying seasonality and decided that data revealing annual variation in
vegetation growth and therewith primary productivity patterns, would be an excellent means to examine spatial and temporal variation in seasonality in terrestrial environments. Apparently, there are a substantial number of studies that have used remotely sensed vegetation (NDVI) data to examine seasonal patterns on regional as well as global scales (e.g. Reed et al. 1994, Moulin et al. 1997, Pettorelli et al. 2005, Bradley et al. 2007, Jonsson et al. 2010, Atkinson et al. 2012, Brown et al. 2012, Eastman et al. 2013). Nevertheless, I had the feeling that an analysis that captures and discusses the ecologically most relevant dimensions of seasonality was missing. Furthermore, discussions of preliminary findings with several researchers who work in this field revealed that my analysis was indeed novel and likely to be very insightful (see research partly stimulated by the results and methods of Chapter 2: Appendix 7.1). The analysis of Chapter 2 shows how biological seasonality is distributed across the globe (Fig. 2.1) and how these seasonal patterns have changed over the last three decades reveal many new insights. One very pertinent aspect of this analysis showed that seasonality has been subjected to significant changes that follow the trends of global warming and increased human activities such as agricultural intensity (see also MacDonald et al. 2011). It also reinforces previous conclusions that colder environments such as the Arctic tundra and taiga experience the most severe changes in both phenology (start of the growing season) as well as in the magnitude of annual variations in bioproductivity (amplitude in seasonal variation; Fig. 2.3). Almost all seasonal habitats have shown evidence of change over the past 30 years. However, the direction of change varied consistently. Thus, the less seasonal habitats that typify the southern hemisphere showed a general decrease in the strength of the seasonal signal, whereas the already highly seasonal habitats from the temperate forests to the Arctic tundra have experienced further increases in their seasonality (Fig 2.2). Changes in seasonality have potential consequences for organisms living in seasonal habitats. Up to now, most studies focusing on the effects of climate change on seasonality and its impact on life have examined the response of animals to changes in phenology - the timing - of the growing season (Parmesan 2006 and references therein). I believe that the results of Chapter 2 provide a means to examine seasonal effects on animals more completely, by considering how both the phenology and the shape of seasonality affect the functioning of life on a global scale. However, the findings also highlight that due to the great heterogeneity in seasonal patterns across the globe and in the changes therein, research from a whole range of different
biomes and from the northern as well as the southern continents is required to improve our predictions of how seasonality will affect patterns of e.g. species distribution, extinction rates, and biodiversity.

Previous studies dealing with infectious disease dynamics often considered seasonality as an on-off situation, largely ignoring the vast differences in seasonality across the globe. These differences might for instance be exemplified by comparing temperate broadleaf forests, which show pronounced seasons with high biomass productions during spring and summer, to Arctic environments, where plant growth is restricted to a few weeks annually. Both of these are seasonal habitats, but they obviously experience vastly different dynamics. In Chapter 3 I hypothesised, that variance in seasonal dynamics will affect the presumed drivers of local AIV infections and I and my colleagues thus predicted that we should see marked differences in the prevalence patterns between locations having different seasonal dynamics. The AIV-Duck system of the North American continent is probably the only infectious disease system that has sufficient cumulative data over a sufficiently large geographical region to test this hypothesis. Although these data still required statistical manipulation, i.e. clustering, to derive adequate sample sizes for regions that differed in seasonal pattern, we found convincing support for our hypothesis. The results showed that while all regions experienced a peak of infection in the late summer, regions experiencing profound environmental variation showed higher peaks and a greater number of total infections compared to regions with lower seasonal variation. These findings suggest that a specific set of biological drivers, in this case the influx of naïve juveniles, host abundance, and seasonally affected variation in the proportion of susceptible adults (Fig 3.1), was able to generate vastly different seasonal infection dynamics depending on the environmental context (Fig. 3.4). This is indeed a significant new finding that will greatly assist in the development of models predicting risks and severity of disease outbreaks in wildlife populations. Such models can ultimately be used to assess the likelihood of spill-over (or spill-back) effects between wildlife and poultry. These results also show that an explicit consideration of geographic variation in seasonality and not simply proxies like latitude, provides a far more powerful framework for understanding the dynamics of infectious diseases.
Although bird migration has been discussed in the context of the results of Chapter 3, it was not included explicitly in the explanation of the analysed seasonal AIV infection dynamics. The patterns described in Chapter 3 play out on a large “macro-ecological” scale across the North American continent. Those patterns might potentially be substantially modified on a local scale by seasonal variation in host aggregation due to migration. One prominent example is the aggregation of Ruddy turnstones (*Arenaria interpres*) feeding on horseshoe crab eggs in Delaware Bay during spring migration. These intense aggregations result in extremely high levels of AIV transmission and prevalence among the turnstones (Krauss et al. 2010). Other local surveillance studies have also shown that host aggregations during migration significantly influences AIV prevalence (van Dijk et al. 2014a). The exact mechanisms of the observed infection patterns and at what point migrants become a significant player in the local infection dynamic is mostly unknown. In order to shed some light on this issue, I collaborated with researchers that have been collecting data of AIV infections at a very local scale (a duck decoy or catching pond in the Netherlands), for an entire year and from a single-species system – the mallard duck (*Anas platyrhynchos*) (van Dijk et al. 2014a). The aim of Chapter 4 was to incorporate different possible mechanisms of AIV infection into mathematical models and then apply model-fitting procedures to evaluate their suitability in explaining the observed prevalence. Mathematical models offer valuable tools for synthesizing information that potentially explains and predicts often complex epidemiological patterns. The first set of mechanisms I tried to fit to the observed prevalence pattern dealt mainly with fine-scale differences in the physiology of the birds. This model assumed for instance that migrants are immunocompromised after migration and arrival at a stop-over site. It quickly turned out, that other factors such as the demographic patterns of migrant and resident mallards had to be included in the model to improve its predictive power. I therefore decided to take a step back and adapt my modelling framework to further investigate a range of mechanisms that could potentially impact the dynamics observed in the duck decoy. The five mechanisms that were evaluated included the presence or absence of a pronounced birth pulse, short-term versus long-term immunity, different proportions of susceptible versus infectious versus recovered migrants arriving at the decoy, different susceptibility of migrants compared to residents and a parameter termed the migratory replacement function (Fig 4.1). The results of the model-fitting procedure indicated that the demography, notably the demographic patterns
of migrants, are indeed the most important drivers of the observed infection dynamic. In this particular system the demographic patterns of migrants seem exceptionally strong and by optimising the parameters for the migrant replacement function, all other mechanisms that were considered became uninformative.

This study provided valuable insight by identifying the importance of migrant replacement on disease transmission. Thus, during peak migration, the replacement of migrating birds by later arriving migrants results in the constant turnover of new migrants and their exposure to resident birds. Because these new arrivals are likely to be susceptible to the local AIV strains, they can thus amplify the local transmission dynamics, leading to pronounced infection peaks during late summer and early autumn as observed by Krauss et al. (2010) and van Dijk et al. (2014b). This pattern of variation in migratory schedules and route can be described in terms of how connected individuals and populations are during their migratory journey. This has been formalised by Webster et al. (2002), who coined the concept of “migratory connectivity” that describes the geographic linkage of individuals and populations across life-cycle stages. This concept has been enthusiastically embraced by the scientific community. Most studies, however, use this term to simply describe the spatial connectivity between individuals of one or multiple populations at their breeding or major overwintering sites. In part stimulated by the results from the mallard simulation study, we argue in Chapter 5 that this concept can be greatly expanded and used in an explanatory and predictive framework that includes the entire annual migratory cycle and its associated spatial and temporal patterns. The two dimensions together have consequences not only for migrant fitness and the dynamics of their populations but also for gene flow, and obviously for local infection dynamics in host-pathogen interactions. Besides the empirical support from Chapter 4, our dynamical network modelling approach also showed that prevalence levels at stopover sites differ significantly depending on degree of synchrony in migration, with those migrating completely synchronously having very different consequences for the local infection dynamics compared to those with a more spread-out migration pattern.

How can we use this framework to explain the results of Chapter 4, the AIV infection dynamics in resident and migrant mallard ducks? Migratory mallards visiting the Netherlands during their southward migratory journey come from vastly different geographical regions (Scott and Rose 1996). These different
populations often show different phenologies in their migration schedule, due to both different migration distances as well as different seasonal dynamics on the breeding grounds. This leads to a low degree of migratory connectivity within the mallard duck population of the East-Atlantic Flyway as a whole. The migrant replacement function used in Chapter 4 reflects exactly that, of a migrating populations with low migratory connectivity. Given the importance of this replacement function in predicting the observed prevalence dynamic and the general structure of the flyway, lend credit to our conclusion that in the replacement function we have captured a major driver of the AIV infection dynamic for that particular site. We can test the generality of this relation by evaluating how well it accounts for the difference in the AI infections within the duck populations of the North American continent. In contrast to the East-Atlantic Flyway where migration of waterfowl is channelled in central-northern Europe (Baltic Sea and Wadden Sea) creating a migratory bottleneck (Scott and Pose 1996), migration on the North American continent occurs along a broad front from north to south with little longitudinal mixing (Lam et al. 2012). This restricts the origin of migrants that visit temperate stopover locations to a relatively small area to the north. This shows that patterns of migratory connectivity can differ geographically from local scales up to the boundaries of major migratory flyways and that the consequences associated with migratory connectivity (Chapter 5) may also differ depending on the location and their larger geographical context. Delaware Bay is again a prime example that on the one hand shows a local infection pattern that does not fit the larger macro-ecological context of North America (see Chapter 3). Yet, on the other hand, their infection dynamic can easily be explained by the local situation and the high degree of spatial and temporal migratory connectivity of the wader and duck populations that visit the Bay during northward migration (Krauss et al. 2010, Bahl et al. 2013).

We have benefited from the immense surge in the surveillance of wildlife disease dynamics, particularly in the AIV system (Hoye et al. 2010), which has provided the opportunity to investigate the ecological mechanisms of small-scale and large-scale infection dynamics. We have found that in order to understand infection dynamics of wildlife populations surveillance studies require both the monitoring of the prevalence as well as the underlying demographic patterns. When it comes to the interface of infectious disease dynamics and long-distance migrating species, one of the biggest challenges is
to follow their often long-distance movements. This problem is further exacerbated by the need to have a large sample size of individual movement data to overcome the inherent inter-individual variation in migratory movement and the often low prevalence levels (Hoye et al. 2010). The ongoing advancements in tracking technologies have already increased our ability to track a wide range of species. However, the weight of accurate GPS devices and the comparatively high costs still hamper our ability to obtain the necessary sample sizes and to successfully track the many smaller-sized migratory species (Bridge et al. 2011, Bridge et al. 2013). For these reasons, light-level geolocators have been welcomed enthusiastically by the research community. This relatively low-cost and lightweight technology arguably provides tremendous opportunity to explore migratory pathways of a range of species. However, the method is not without its shortcomings. Among these shortcomings is the need to recapture birds after they have carried a device throughout a migration cycle and then to accurately deduce the geographic positions in relation to the recorded time based on light intensity recordings. Because these are susceptible to any shading that may have affected the light intensity received by the sensor of the tag (Lisovski et al. 2012), the data must be scrutinised carefully to avoid miscalculations. Even after excluding or processing what appears to be spurious data, one must consider whether the location estimates provide the required spatiotemporal resolution to test particular hypotheses. The excitement in using these loggers without being aware of the assumptions underlying their application in deducing location has led to many false hopes about their application and to erroneous interpretations of the tracking data (see Appendix 7.2). In Chapter 6 I investigated the capabilities of a more sophisticated analytical approach that I developed over the last three years in cooperation with two colleagues (MCMC path estimates: Wotherspoon et al. 2013). The chapter includes a comparison with a tool I developed previously that provides basic low-level analysis (simple threshold analysis: Lisovski and Hahn 2012).

Besides new insights into the migration pattern of Sanderlings (*Calidris alba*) along the East-Asian-Australasian Flyway, the geolocator analysis highlights the advantage of more sophisticated analytical tools for location estimates. The developed software called ‘SGAT’ (R package: Wotherspoon et al. 2013) uses Markov Chain Monte Carlo (MCMC) simulations that provides a framework that enables additional information, such as species-specific habitat preferences, movement behaviours, and home range, to be incorporated. This greatly
reduces the probability of erroneous, incompatible location estimates. Moreover, the method allows estimation of confidence intervals for location estimates that reflect the quality of the data. Finally, the method permits the estimation of a continuous path, whereby each observation is used and evaluated in relation to all other observations, rather than the relatively arbitrary qualification of each position in isolation, and hence the potential omission or inclusion of positions derived by simple threshold estimates (Sumner, Wotherspoon et al. 2009). Those features are crucial for the analysis of individual migration paths using any tracking technology with an associated uncertainty and even more for inferring population level movements that are required to describe e.g. migratory connectivity pattern and the consequences for e.g. population dynamics, gene flow and host pathogen interactions such as infectious disease dynamics.
Appendices

Appendices to Chapter 2

A.2.1. Extracting seasonal features from satellite data

This document is meant to support the description of the method section by illustrating the results of the various steps starting with the normalized difference vegetation index (NDVI) and ending with the extraction of the following seasonal features:

1. Strength of the seasonal signal
2. Amplitude of the reproductive season
3. Onset of the reproductive season
4. Termination of the reproductive season
5. Duration of the reproductive season

The example location and the raw data

For the sake of illustration, a random location was chosen (Figure A.2.1.1) and the various steps that had been applied to the global dataset were run for this location only. The used NDVI time series contains global measurements (except Antarctica and northern latitudes above 75.3 degrees) at a 16x16km spatial and a weekly temporal resolution. The used snow cover data to correct the NDVI observations has identical temporal resolution but a 25x25km spatial resolution.

Figure A.2.1.1: Map with the location chosen to illustrate the process of extracting seasonal features for our analysis on seasonality and its changes over a 30 year period.
resolution. We thus resampled the snow cover data to match the NDVI spatial resolution. Illustration of the raw NDVI and the corrected NDVI values for the example location are sown in Figure A.2.1.2. The spatial resolution of the NDVI data was decreased to a 57.2x70.8 km aggregate grid-cell, which resulted in collapsing 16 original cells to one cell. The mean of these 16 cells was used for the power analysis and all 16 time series were used to extract an average AoS, SoS and Eos for each of the aggregate grid-cells.

Figure A.2.1.2: Snow corrected NDVI values for the selected position. Red points represent weeks with snow cover: NDVI values for those weeks were corrected to zero.

Figure A.2.1.3: a) The power matrix over periodicity and time (excluding the ‘cone of influence’. b) The power over periods for each year separately and indicating the significant power sections (red). c) The power of the overriding periodicity (in this case only annual periodicity was detected) over the years and the linear trend line.
Wavelet analysis
To define the periodicity and the statistical strength of the seasonal signal we applied a wavelet analysis to the snow corrected NDVI time series (Figure A.2.1.3). Wavelet analysis was performed using the wt function from the R package biwavelet, using all default settings, including ‘morlet’ as the mother wavelet (Gouhier 2014).

A curve fitting procedure to derive seasonal features from NDVI time series
Negative asymmetric double-sigmoid curves (ADS) were fitted to the data using Maximum Likelihood. The fitted curve enables the extraction of phenological metrics such as the start and the end of the growing season (SoS, EoS). The following steps were applied to the 16 time series of each grid cell with significant annual periodicity derived by the power analysis:

1. A cosine function was fitted to the time series allowing to separate years based on the underlying cyclic variation (Figure A.2.1.4a).
2. A positive ADS curve was fitted to the years split by the minimums of the fitted cosine curve (winter-winter years) (Figure A.2.1.4b).
3. A negative ADS curve was fitted to the years split by the maximum of the fitted cosine curve (summer-summer year) (Figure A.2.1.4c).
4. The smooth transitions between the two curves were calculated, resulting in the ‘global’ ADS curve (Figure A.2.1.4d).
5. The seasonal amplitude was extracted by subtracting the NDVI minimum (0.5 quantile) from the NDVI maximum (0.95 quantile) (Figure A.2.1.5).
6. The onset and the termination of the season for each year were defined as the date at which the global ADS curve exceeded 15% in spring and fell below 15% of the ADS amplitude in autumn (Figure A.2.1.5).
7. The duration of the reproductive season was calculated as the time difference between onset and termination of the season.
Figure A.2.1.4: Asymmetric double-sigmoid curve (ADS) fitting. a) Step 1: fitted cosine curve for subsequent split of the time series in winter-winter years (blue to blue points) and summer-summer years (red to red points). b) Step 2: positive ADS curve for winter-winter years. c) Step 3: Negative ADS curve for summer-summer years. d) Global ADS curve based on negative and positive curve with smooth transition between the overlap.
To investigate trends in the seasonal features a linear model was applied to the time series of the extracted seasonal features.

Figure A.2.1.5: The global ADS fit (violet curve) for four years, and the extracted seasonal features: Minimum and maximum NDVI (horizontal dashed lines) and onset/termination of the reproductive season (vertical dashed lines).

Figure A.2.1.6: The extracted seasonal features over the years: a) Seasonal Amplitude, b) Onset of the reproductive season (SoS – Start of Season), c) Termination of the reproductive season (EoS – End of season) and d) Duration of the reproductive season (DoS). The solid lines are trend lines from a linear model.
A.2.2. Strength of seasonal signals: the influence of amplitude, noise and predictability

This appendix aims to illustrate the effect of different seasonal dimensions on the power spectrum of the wavelet analysis that was used to describe the strength of the seasonal signal. In general, wavelet analysis decomposes a time series into time-frequency space, enabling the determination of both the dominant modes of variability and how those modes vary over time (Torrence and Compo 1998).

Here, we simulated theoretical seasonal time series with difference in the amplitude of the growing season (Figure A.2.2.1a), potential bias (signal to noise ratio; Figure A.2.2.1b) in the NDVI data due to differences in the vegetation covers and in the consistency of the seasonal phenology (onset and termination of the reproductive season; Figure A.2.2.1c). The letter was simulated by independently changing the mean of each annual growing season to a certain extent forwards or backwards.

Figure A.2.2.1: Simulated seasonal variations with changes in the amplitude (a) the noise (b) and the consistency in the phenology of the seasonal variation(c).
We then run the wavelet analysis using the theoretical NDVI curves and plotted the results (power of the periodicity or the wavelet spectrum) on two-dimension heat maps (Figure A.2.2.2).

The results show that the power of the periodicity is most sensitive to the amplitude of the seasonal variation (Figure A.2.2.2 a-b). The signal to noise ratio had no effect on the power (Figure A.2.2.2 a) however the consistency of the phenology did negatively affect the power of the periodicity (Figure A.2.2.2 b). The results show, that although the amplitude of the growing season has the most significant effect on the power of the seasonality and e.g. the strength of the seasonal signal, changes in the phenology between years may additionally modulate the results. We therefore conclude, that the wavelet analysis adds important information and is not just another presentation of the amplitude of the seasonal signal (AoS).
Appendix to Chapter 3

Table A3.1 Number of individuals per species per cluster used in the analysis

<table>
<thead>
<tr>
<th>Host species</th>
<th>Cluster 3</th>
<th>Cluster 2</th>
<th>Cluster 1</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard* Anas platyrhynchos</td>
<td>6337</td>
<td>10141</td>
<td>5818</td>
<td>22296</td>
</tr>
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* Contains 46 Anas platyrhynchos x Anas sp. hybrids
A.3.2 - Sampling distribution 2006 – 2011

Figure A3.2_1: Number of reported samples (IR Database: http://www.fludb.org) from dabbling ducks for the United States of America and Canada within the years 2006 – 2011.

A.3.3 SIR model sensitivity analysis (gamma)

Figure A3.3_2: Force of infection curve for different $\gamma$ values; a) $1/3$ day$^{-1}$ and b) $1/13$ day$^{-1}$. Dashed line indicates the FOI curve with the mentioned $\gamma$ values, whereas the colour lines show the curve with $\gamma = 1/8$ day$^{-1}$ as used in the model of the main text.
A.3.4 SIR model sensitivity analysis (prev0)

Figure A3.2_3: Force of infection curve for different initial prevalence levels; a) 2.5% and b) 5%. Dashed line indicates the FOI curve with the respective initial prevalence, whereas the colour lines show the curve with 0% initial prevalence as used in the model of the main text.
Appendices to Chapter 4

A.4.1 Model equations

The ordinary differential equation for the background model (eq. 1-6) are shown in the main text. Here, the structural changes that occurred due to one of the five model modifications are shown.

1. Birth pulse

Resident adult population:
\[
\frac{dS_R(t)}{dt} = -\beta(I_R + I_M)S_R + \sigma R_R - \eta S_R - mS_R
\]
\[
\frac{dI_R(t)}{dt} = \beta(I_R + I_M)S_R - \gamma I_R + \eta S_R - mI_R
\]
\[
\frac{dR_R(t)}{dt} = \gamma I_R - \sigma R_R - mR_R
\]

Resident juvenile population:
\[
\frac{dS_R(t)}{dt} = -\beta(I_R + I_M)S_R + \sigma R_R - \eta S_R - m_j S_R + B(0.5 \cdot N_{pop}) N_{pulse}
\]
\[
\frac{dI_R(t)}{dt} = \beta(I_R + I_M)S_R - \gamma I_R + \eta S_R - m_j I_R
\]
\[
\frac{dR_R(t)}{dt} = \gamma I_R - \sigma R_R - m_j R_R
\]

Migrant population:
As eq. 4-6

3. Susceptible migrants

Resident population:
As eq. 1-3

Migrant population:
\[
\frac{dS_M(t)}{dt} = -\beta(I_R + I_M)S_M + \sigma R_M - \eta S_M - mS_M + M N_{pop} P r_m
\]
\[
\frac{dI_M(t)}{dt} = \beta(I_R + I_M)S_M - \gamma I_M + \eta S_M - mI_M
\]
\[
\frac{dR_M(t)}{dt} = \gamma I_M - \sigma R_M - mR_M
\]
4. Differential susceptibility

**Resident population:**
\[
\frac{dS_R(t)}{dt} = -\beta_R - (I_R + I_M)S_R + \sigma R_R - \eta S_R - mS_R + B(0.5 \cdot N_{pop})N_{hatch}
\]
\[
\frac{dI_R(t)}{dt} = \beta_R(I_R + I_M)S_R - \gamma I_R + \eta S_R - mI_R
\]
\[
\frac{dR_R(t)}{dt} = \gamma I_R - \sigma R_R - mR_R
\]

**Migrants:**
\[
\frac{dS_M(t)}{dt} = -\beta_M(I_R + I_M)S_M + \sigma R_M - \eta S_M - mS_M + M\left(\frac{S_R}{S_R + I_R + R_R}\right)N_{pop}P_{r_m}
\]
\[
\frac{dI_M(t)}{dt} = \beta_M(I_R + I_M)S_M - \gamma I_M + \eta S_M - mI_M + M\left(\frac{I_R}{S_R + I_R + R_R}\right)N_{pop}P_{r_m}
\]
\[
\frac{dR_M(t)}{dt} = \gamma I_M - \sigma R_M - mR_M + M\left(\frac{R_R}{S_R + I_R + R_R}\right)N_{pop}P_{r_m}
\]

4. Replacement of migrants

**Resident population:**
As eq. 1:3

**Migrants:**
\[
\frac{dS_M(t)}{dt} = -\beta_M(I_R + I_M)S_M + \sigma R_M - \eta S_M - mS_M
\]
\[
+ M\left(\frac{S_R}{S_R + I_R + R_R}\right)N_{pop}P_{r_m} + R(I_m + R_M)
\]
\[
\frac{dI_M(t)}{dt} = \beta_M(I_R + I_M)S_M - \gamma I_M + \eta S_M - mI_M
\]
\[
+ M\left(\frac{I_R}{S_R + I_R + R_R}\right)N_{pop}P_{r_m} - R(I_m)
\]
\[
\frac{dR_M(t)}{dt} = \gamma I_M - \sigma R_M - mR_M + M\left(\frac{R_R}{S_R + I_R + R_R}\right)N_{pop}P_{r_m} - R(R_m)
\]
### A.4.2. Model fitting results

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Appendix to Chapter 5

A5.1 Model description

We developed a simple model to illustrate how disease dynamics may change when explicitly considering, and varying, the phenology and synchrony of migration. To this end, we combined a dynamic network model with an epidemiological and a migration model such that infection status and location of individuals (‘nodes’ in the network) could change as a result of infection dynamics and migration, respectively. We followed disease prevalence over time and location.

Network dynamics. In the model, individuals are defined as nodes in the network and individuals can be connected via links (‘edges’). We used a dynamic network, in which links between individuals can form and dissolve over time; yet, the average number of edges per node in the network (i.e. the mean degree) is preserved. Thus, the model incorporates time-varying contact structures. We assumed a relatively sparse, undirected network with a mean degree of 0.5; links lasted on average 5 time-steps. We used a temporal exponential-family random graph model that provides a method of constructing networks with a given set of properties. Markov Chain Monte Carlo can then be used to create a range of plausible networks that agree with a wide variety of information collected on network structures even if the complete network is unknown. We used the statnet suite for constructing our network models (Handcock et al. 2003), for details see (Goodreau et al. 2008) and (Handcock et al. 2008), and its sub-package ‘EpiModel’ (Jenness et al. 2014) for the epidemiological network model and R 3.1.1. for all further analyses and scenarios (Team 2014).

We defined a network consisting of 200 individuals (nodes). In addition to changing links, individuals could migrate and get infected or recover – as a consequences of infection dynamics and migration.

Infection dynamics. We used a SIS (susceptible-infectious-susceptible) model (Keeling and Rohani 2008), in which susceptible individuals can be infected with probability, $\tau$, if connected to an infected individual. Infected individuals recover with probability $\gamma$, and re-enter the pool of susceptibles. Although we had no particular parasite or disease in mind, we used parameter values of $\tau = 0.2$ and $\gamma = 0.07$, i.e. infections lasted on average 14 days.

Migration. We consider a simple type of migration from a starting to a destination site. Migration is instantaneous and depending on specific scenarios (see below), individuals migrated at or around a mean migration date,
\[ t_{mig} = 100. \] As we assume the two sites to be distant, no links (and thus, no parasite transmissions) were allowed between individuals at disparate sites. For simplicity, we assume a time-step of one day; and we followed disease dynamics over 200 days. At the start of each simulation, all individuals were located at the starting site and 10% of the individuals were randomly infected. As the model included various stochastic components, e.g. formation and dissolution of links, parasite transmission and recovery from infection, we ran 100 repetitions of each scenario and analysed the average prevalence per site as well as its 25% and 75% quantiles (not shown).

### A5.2 Scenarios

We explored the role of migration synchrony and phenology on disease dynamics in two sets of scenarios: First, we investigated the gradient from fully synchronous to asynchronous migration by increasing the standard deviation around the mean migration date, \( t_{mig} = 100, \) from \( \sigma = 0, 1, 2, 5 \) to 10. The reasons for such variations in migration dates are not further specified here but could be due to, e.g. variation in fuelling rates (Seewagen et al. 2013), sex-specific constraints and selection pressures (Saino et al. 2010), differential use of environmental factors that trigger departure (Gordo et al. 2013, Otero et al. 2014), or a delayed departure of infected individuals. The latter might result from disease symptoms such as fatigue, lowered activity, slower fuelling (Bradley and Altizer 2005, van Gils et al. 2007a).

Secondly, we explored changes in the phenology of parasites relative to migration phenology. To this end, we introduced a period of increased (environmental) parasite pressure (or increased susceptibility to infection) at one site and varied the onset of this period relative to migration. We used a period of 20 days of increased parasite pressure; and this period started at day \( t = 60, 90, \) or 120 at the starting site such that it was either distinctly before or distinctly after migration, or individuals migrated in the middle of this period. During this period, individuals at the starting site were additionally infected with a probability of 0.5.

Increased parasite pressure or susceptibility may result from various processes: Individuals using parasite-rich habitats within a site (Hoye et al. 2012a), or the aggregations of immense numbers of migrants at key refuelling locations en route enhance parasite transmission and thus, increase prevalence (Krauss et al. 2010). Some studies have also suggested periods of increased susceptibility during the annual cycle in migratory animals resulting from, e.g. seasonal
variations in immune function (Buehler et al. 2008) or the intense physiological demands of migration that may trade off with immune responses (Gylfe et al. 2000).

A.5.3 Results
For scenarios on changes in synchrony, we found prevalence to remain at the same level on both starting and destination site in an entirely synchronously migrating population. However, if individuals migrated asynchronously, i.e. spread out over time, prevalence gradually decreased at the starting site and gradually increased at the destination and thus, differed considerably for a long period (Fig. 5.3a-b).

Changing the onset of elevated parasite pressure also affected prevalence. While prevalence obviously was unaffected when parasite pressure was increased after all individuals had migrated to the destination site, prevalence in the population at the starting site was elevated when parasite pressure was elevated before and even more so, if it coincided with migration. These variations in prevalence at the starting location then spilled over to, and influenced, prevalence at the destination (Fig. 5.3c-d). At the destination site, prevalence subsequently decreased towards a low level (which is determined by epidemiological parameters and average number of links, see above).

Thus, both migration synchrony and phenology importantly shaped (local) disease dynamics (Fig. 5.3) and prevalence varied widely with alterations in the phenology or synchrony of migration even though the underlying epidemiology was kept constant.

Although the model presented here is on purpose kept simple, its findings have implications for the spread of diseases – if we want to estimate in how far migratory animals contribute to the long-distance spread of parasites and pathogens, we need to be specific about the timing of migration.

Obviously, there are many more issues that could be explored with such epidemiological model, including applying it to specific pathogens and parasites, to a variety of migratory animals with more complex migrations and thus, testing particular assumptions on (changes in) contact structures, and interactions between synchrony and phenology in combination with demographic processes.
Appendix to Chapter 6

A.6.1. Sexing of B013 via Principal Component Analysis

This supplement illustrates the method used to sex B013 by principal component analysis (PCA) using morphological measurements from all 14 tacked Sanderlings.

Table 6.1.1: Morphological measurements of all 14 tracked Sanderlings. The sex of all individuals (1 = Female, 2 = Male), except for B013 was determined using molecular analysis.

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Figure 6.1.1: Right panel shows the variance explained by the three principal components (PCs) and the loadings of the three input variables (Culmen = CL, HB = Head-Bill, WL = Wing length). Left panel shows the first PC by sex and the position of Sanderling B013 along the first PC.
Appendix to Chapter 7

A7.1: A long-distance migrant pays the bill for Arctic warming while wintering in the tropics

This appendix has been included with consent of the principal author.

Jan A. van Gils¹, Simeon Lisovski², Włodzimierz Meissner³, Tamar Lok⁴,⁵, Agnieszka Ożarowska³, Jimmy de Fouw¹, Mikhail Y. Soloviev⁶, Theunis Piersma¹,⁴ & Marcel Klaassen²

Abstract

Reductions in body size have recently been classified as a universal response to climate change. We here present evidence for a non-adaptive case of such body shrinkage, potentially due to malnutrition in early life. We show that an avian long-distance migrant (red knot Calidris c. canutus) experiencing globally unrivaled warming rates at its High-Arctic breeding grounds, produces smaller offspring with shorter bills during summers with early snowmelt. At their tropical wintering grounds, short-billed individuals eat fewer deeply buried bivalve prey and forage on shallowly buried seagrass rhizomes instead. However, this alternative diet cannot prevent reduced survival among these smaller birds. This is the first study showing that a seasonal migrant pays the price of warming conditions in the Arctic at its tropical wintering destination.

Main text

Phenological changes and geographical range shifts represent phenotypic responses to global climate change (Parmesan and Yohe 2003). Only recently discovered, but already considered as the third universal phenotypic response to global warming (Gardner et al. 2011), is shrinkage of body size (Sheridan and Bickford 2011, Baudron et al. 2014, Teplitsky and Millien 2014). Smaller bodies may be adaptive as they are considered advantageous in warmer climates (Bergmann’s rule (Teplitsky and Millien 2014)). Nevertheless, non-adaptive explanations for this phenomenon have also been put forward: climate change may disrupt trophic interactions, potentially leading to malnutrition during an organism’s juvenile life stage (Teplitsky et al. 2008, Rode et al. 2010) and poor growth that cannot be compensated for later in life (Metcalfe and Monaghan 2001). To tell adaptive from non-adaptive causes, we need the documentation...
of direct links between components of fitness and climate-induced variations in body size.

Figure A.7.1.1: (A) Over the past 31 years, snow at the red knots’ (Calidris canutus canutus) High-Arctic breeding ground at Taimyr Peninsula has been melting progressively earlier at an average rate of 0.5 day/year (plotted are the dates at which 1/3 of the entire breeding range became snow-free; $R^2 = .29, F_{1,28} = 11.71, P < .005$). (B) Juvenile red knots, captured during brief stopovers in Poland on their first southward migration from the Arctic, had lower body masses after breeding seasons in which snow had disappeared early ($R^2 = .39, F_{1,22} = 14.29, P < .005$; each dot denotes annual mean with number in dot giving year). (C) Moreover, they also had shorter bills when in the Arctic snow melted earlier ($P < .01$; each dot denotes annual mean), especially in years when local vegetation greenness was low ($P < .005$; taking breeding-ground NDVI, indicated by greenness of the symbols, as a proxy). Together, snowmelt date and NDVI explained 38% of the variation in annual average bill length ($F_{2,20} = 6.25, P < .01$).
Although we speak about global warming, some parts of the globe are warming up faster than others. Notably in the Arctic, warming occurs at unprecedented rates (Tingley and Huybers 2013), a process called ‘Arctic amplification’ (Screen and Simmonds 2010). On this basis body size reductions would be expected to be most pronounced in the world’s most northerly region (Rode et al. 2010). Many Arctic-breeding avian species are long-distance migrants spending the northern winter at lower latitudes where climate changes are less strong or even absent. Hence, the costs of having a smaller body at the climatologically more stable wintering grounds (where they spend most of their time Leyrer et al. 2013) might outweigh any benefits on the breeding grounds.

We here show that over the past 31 years snowmelt has occurred progressively earlier in the High-Arctic breeding grounds of a shorebird (red knot; *Calidris canutus canutus*) at Taimyr Peninsula (Fig. 1; 76-78°N), at a rate of about half a day per year (Fig. 7.1.1 A; \( R^2 = .29, F_{1,28} = 11.71, P < .005; \) based on the analysis of MODIS satellite images). During these three decades, 1,904 juvenile red knots were caught and their body sizes measured at a stopover in Poland, during their first southward migration to the West-African nonbreeding,

![Figure A7.1.2: (A) Based on the analysis of stable isotopes, we conclude that juvenile red knots (\( N = 666 \) birds) largely ignore the most abundant but mildly toxic prey, *Loripes lucinalis*. However, with an increase in age, both immature (\( N = 150 \)) and adult red knots (\( N = 1,671 \)) added significant amounts of *Loripes* to their diet, but only so in birds with long bills. (B) This bill-length dependent diet shift may be explained by the depth distribution of *Loripes*, with the majority of these bivalves living between 30-40 mm, i.e. precisely the range of bill lengths. The other two food sources, *Dosinia isocardia* and *Zostera noltii* rhizomes, are found at shallower depths accessible to all red knots.](image-url)
wintering grounds. Our analyses revealed that these juvenile birds were on average smaller after Arctic summers with an early snowmelt, notably with respect to body mass (Fig. 2B; $R^2 = .39, F_{1,22} = 14.29, P < .005$) and bill length (Fig. A.7.1.1 C; $R^2 = .38, F_{2,20} = 6.25, P < .01$). Significant reductions in tarsus and wing length were also best explained by date of snowmelt. The best model explaining annual bill length variation additionally included breeding-ground NDVI (Normalized Difference Vegetation Index, in this case expressed as the area under the fitted seasonal trend, being a proxy for total primary biomass production), with longer-billed birds captured after summers with high NDVI values (Fig. A.7.1.1; $P < .005$). These annual size variations are still apparent once juveniles arrive at their main wintering quarter on the Banc d’Arguin, Mauritania (annual average juvenile bill length in Poland and Mauritania correlate strongly: Pearson’s $r = .74$), where red knots show no signs of compensatory growth (body size dimensions, including bill length, are highly repeatable within individuals caught twice at Banc d’Arguin, see Fig. A.7.1.4). Regardless of the causes of having fledged with a smaller body in summer, the consequences of a short bill during the nonbreeding season may be profound. Red knots use their long tapered bills to detect and retrieve buried mollusk prey from intertidal sediments (Piersma et al. 1998). On the basis of stable-isotope

![Figure A7.1.3.: Annual survival rate (± SE) increases as a function of bill length in juveniles ($N = 671$ birds), while this relation is absent in adults ($N = 1,981$ birds; distinguishing between survival in the first year after capture (adult 1) and later (adult 2+)). Estimates plotted here are for survival from late 2012 to late 2013 (but note that the bill-length effect on juvenile survival is found in each year). Line (± shaded area) give regression fit (± SE).](image-url)
analyses of 2,487 birds caught on the Banc d’Arguin we conclude that short-billed individuals do not tend to rely much on the most abundant bivalve prey species, *Loripes lucinalis* (*Loripes* from now on), but longer billed birds do (Fig. A.7.1.2; $R^2 = .18$, $F_{3,2483} = 182.42$, $P < .00001$). This is easily explained by most *Loripes* being buried out of reach of a short-billed knot: an individual with a 30-mm long bill is able to access about 1/3 of all *Loripes*, while a 40-mm long bill has access to about 2/3 (Fig. A.7.1.1.3). Shorter-billed red knots consume the more accessible bivalve *Dosinia isocardia* (*Dosinia* from now on) and the shallow rhizomes of seagrass *Zostera noltii* (*Zostera* from now on). It is important to note that only after the birds’ first winter the consumption of *Loripes* is increased ($P_{\text{age:bill interaction}} < .00001$). This is likely due to the fact that *Loripes* is mildly toxic, causing diarrhea in the consumer (Oudman et al. 2014), a phenomenon which is due to the sulphide-metabolism of endosymbiotic bacteria living inside this bivalve’s gill (van der Geest et al. 2014). Possibly, juvenile birds need physiological adjustments before they can digest this special type of prey efficiently (Stein et al. 2005). Clearly, only the young birds with the longer bills can make the switch to include the deeply living *Loripes*, the shorter-billed birds thus being stuck with a ‘juvenile diet’ of *Dosinia* and rhizomes. These alternative food sources may not fully compensate for the reduced access to and consumption of *Loripes*. *Dosinia*, although being the highly preferred prey (van Gils et al. 2012, van Gils et al. 2013, Oudman et al. 2014), has an erratic occurrence with a few rich years amongst many poor years (van Gils et al. 2013, Ahmedou Salem et al. 2014). By contrast, although *Zostera* is always abundant in Banc d’Arguin (Wolff and Smit 1990), the rhizomes may be a rather poor quality food (Pírc 1989, Pérez-Lloréns et al. 1991), notably for birds specialized in digesting animal tissue (Piersma et al. 1993b). Indeed, observations under experimental conditions show that red knots only consume rhizomes when bivalves are offered at very low densities (van Gils et al. in prep.). Hence, the inability to access high quality *Loripes* may come at a survival cost.

Based on individual colour-ringing a total of 2,652 red knots during annual expeditions to Banc d’Arguin between 2002 and 2013, and subsequent resighting of these individuals, we show that a short bill is indeed associated with lower survival. However, this is only so for survival from the 1st to the 2nd winter – adult annual survival is unaffected by bill length (Fig. A.7.1.3; bill-length dependent survival in juveniles also explains why juveniles have on average shorter bills than adults). Since date of snowmelt affects bill length (Fig.
A.7.1.1), and bill length affects annual juvenile survival in the tropics, it does not come as a surprise that date of snowmelt affects annual juvenile survival in the tropics (Fig. A.7.1.4; $R^2 = .49$, $F_{1.9} = 8.77$, $P < .05$). On this basis we reject the hypothesis that red knots shrink because smaller individuals are fitter than larger individuals. In fact, unless they produce more offspring, the smallest individuals are least fit. This is consistent with a non-adaptive explanation of climate-induced body shrinkage. It is likely that malnutrition during chick stage causes smaller bodied red knots. Neonatal red knots feed on adult arthropods (Schekkerman et al. 2003) that emerge from a defrosting tundra soil (Danks 1999). With the rapid advancement in the seasonal appearance of High-Arctic arthropods (Høye et al. 2007, Høye and Forchhammer 2008), red knot chicks are likely to face a trophic mismatch by hatching too late relative to the peak insect abundance (Høye et al. 2007, McKinnon et al. 2012). If true, this occurs in spite the evidence for earlier nesting in High Arctic shorebirds (Liebezeit et al. 2014), and in spite the observation that red knot spring migration through

![Figure A.7.1.4: Juvenile annual survival rate (± SE) increases with date of snowmelt. Number in dot gives year and line represents linear regression fit. Note that the best-supported model ($\Phi_{\text{yam:real_age+time p:time+site}}$) estimating survival rate here includes time dependence, which is why survival rate for the last year in the dataset (2013) cannot be estimated as it cannot be separated from resighting rate.](image)

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France is actually advancing (however, at a rate of just 1 day every 4 years; van Gils and Bocher unpub. data).

Besides advancing the timing of the insect peak, earlier snowmelts are also known to depress the peak’s amplitude. This is because earlier snowmelts cause greater soil temperature fluctuations, thereby enhancing mortality among insect larvae (Bale and Hayward 2010). Our result that bills are smaller in years with low breeding-ground NDVI values (Fig. A.7.1.1) hints at the importance of the insect peak’s amplitude, since low NDVI values reflect low insect abundances (Grilli and Gorla 1997).

Negative effects of climate change on growth have been shown before (Rode et al. 2010, McKinnon et al. 2012), but never have the fitness consequences of climate inhibiting growth been quantified (in this case in terms of feeding behaviour and ultimately survival). Our study is the first to show survival repercussion of climate change at the individual level, not to confuse with survival effects of climate change at species level, like population decreases and extinction risks (Cahill et al. 2013). We stress that, in our case, these repercussions are paid outside of the High-Arctic region. Although in this region climate change appears most noticeable for many life forms, for migratory red knots the bill is paid only later, once wintering in the tropics.

**Methods**

**Temperature data**

Daily mean temperatures were obtained for 1983-2013 across the entire breeding range of the *Calidris c. canutus* subspecies (northern Taimyr Peninsula as defined by Lappo et al. (2012b)). Following the methodology outlined elsewhere (Aharon-Rotman et al. 2014), daily temperature data (May-August) were downloaded from the NOAA National Climatic Data Centre (Laaksonen et al. 2006), which were then plotted as temperature surface maps (Manifold 8 GIS system), using ‘gravity’ as the interpolation algorithm, while taking a search radius of 500 km and a maximum of ten weather stations. Subsequently, surface maps were overlaid on the knot’s breeding range, and surface values were averaged across this range, yielding a mean temperature for each day. Next, for each year separately a quadratic model was fitted to these daily mean temperatures (using the lm function in R), and the date at which the increasing part of this fit reached 0 °C was defined as $D_{TO}$. 
Normalized Difference Vegetation Index (NDVI) data

Based on MODIS satellite images, weekly NDVI data on a scale of 16 × 16 km grid cells were downloaded (Zabel et al. 2005) for the period 1983-2013. Next, cells located in the subspecies’ entire breeding range were selected for further analysis. Then, for each year separately, a smoother was fitted through the data (using the loess function in R, span set to 0.3), where after values due to the albedo effect of snow cover were removed: in case the smoother decreased in spring before reaching the summer maximum and increased in autumn then the values before the minimum in spring and after the minimum in autumn were removed. The smoother was then used to determine D1_{NDVI}, i.e., the date at which the fitted NDVI crosses a threshold value of 0 (before reaching the yearly maximum), and to determine A_{NDVI}, i.e., the area underneath the smoother from the start (D1_{NDVI}) to the end (D2_{NDVI}) of the season (with the latter defined as the date at which the fitted NDVI crosses a threshold value of 0.1, after having reached the yearly maximum). A threshold of 0.1 was used for defining D2_{NDVI} since the vegetation index was in general still above 0 at the start of the snow-covered season. Note that data were missing for 2001 and the second half of 1994.

Snow cover data

Based on the remotely sensed NOAA/NCDC climate data on the Northern Hemisphere Snow Cover Extent (Robinson et al. 2012), weekly snow and ice cover data for the period 1983-2013 on a scale of 24 × 24 km were downloaded (Brodzik and Armstrong 2013). Next, grid cells falling in the subspecies’ entire breeding range were extracted. Next, large erratic changes in snow cover during summer were removed as these reflect rather unpredictable incidences and do not reflect the main phenology of the seasonal snowfall-thaw cycle. Then, data were modelled using a maximum likelihood fit (mle2 function from R package bbmle) of the asymmetric Gaussian model function (Jönsson and Eklundh 2002) with a binomial error distribution. Using these year-specific fits, date of snowmelt D_{SM} was then determined as the date that the fitted curve predicted 1/3 of the whole area to be snow free, whereas date of snowfall D_{SF} was determined as the predicted date when 1/3 of the whole area was covered by snow. R code used to extract phenological from temperature, NDVI and snow cover data can be found online: https://github.com/slisovski/vanGils-Appendix.
Body size of juvenile red knots at first stopover (Poland)

Every autumn, between 1983 and 2013 we captured red knots at a stopover site in Poland, Puck Bay (Meissner 2005, Meissner 2007), a site that is mainly used by juvenile red knots (after having left the breeding grounds, adult red knots usually make their first stopover in the Wadden Sea (Nebel et al. 2000)). Birds were captured in walk-in traps, where after they were aged on the basis of plumage (Prater et al. 1977), distinguishing juveniles (1st-calendar-year birds) from adults (> 2nd-calendar-year). Body mass (± 1 g) and structural size measurements were recorded, including length of bill (± 0.1 mm), tarsus (± 0.1 mm) and wing (± 1 mm). Across the entire 31-year period, a total of 2,598 red knots were caught, among which 1,904 juvenile birds. In the analyses we excluded years in which fewer than 10 juveniles were caught.

Body size, diet and survival of red knots at their wintering site (Mauritania)

In Banc d’Arguin (Mauritania) we caught a total of 2,652 red knots across 13 winters (2002-2014), mostly during early winter (November/December), with some birds caught in January (150 birds). Catches were mostly done by using mist-nets, except for one catch for which we used canon-nets (January 2013). Upon capture, body mass (± 1 g) and structural size measurements were recorded, including length of bill (± 0.1 mm), tarsus (± 0.1 mm) and wing (± 1 mm). Furthermore, the birds were aged based on their plumage (Prater et al. 1977), distinguishing between 1st-calendar-year birds (juveniles), 2nd-calendar-year birds (immatures) and older birds (adults).

From the brachial vein a small blood sample (10-100 μL) was taken, which was stored in 70% ethanol. At NIOZ, samples were stored at -80 ºC until analysis. After extracting DNA to molecularly identify each individual’s sex (Baker et al. 1998), leftovers of the samples were used to determine the stable isotope ratios of carbon (13C) and nitrogen (15N). In order to do so, samples were freeze-dried to constant mass (Dietz et al. 2010) before analysed in a Thermo Scientific (Flash 2000) organic element analyser coupled to a Delta V isotope ratio mass spectrometer. A microbalance (Sartorius XM1000P) was used to weigh 0.4-0.8 mg of freeze-dried blood into 5 x 9 mm tin capsules. Isotope values were calibrated to an acetanilide lab standard, controlled for a urea lab standard and corrected for blank tin capsules. In this way we were able to analyse blood samples from most birds (N = 2,487 individuals), which we analysed in random order with respect to year. Stable isotope ratios of the three main food sources
Dosinia isocardia, Loripes lucinalis and rhizomes of Zostera noltii) were taken from Catry et al. (Catry et al. 2015), and the discrimination values from an experimental validation study in which captive red knots were given a monospecific diet consisting either of Dosinia or Loripes (van Gils and Ahmedou Salem 2015). These values were then used by the siarsolomcmcv4 function from the R package siar (Parnell and Jackson 2013) to estimate the relative contribution of each food source to an individual’s diet.

Before release, birds were tagged using a combination of four color-bands and a flag, allowing individual recognition in the field, thereby enabling annual survival rate estimations (Leyrer et al. 2012, Leyrer et al. 2013, van Gils et al. 2013). By intense resighting efforts using telescopes in all 13 nonbreeding seasons (not only in Banc d’Arguin, but also elsewhere along the flyway) we could estimate annual winter-to-winter survival rate across the 12 intervening years. Apparent (or local) survival (Φ) and recapture probabilities (p) were estimated from live encounter data using Cormack-Jolly-Seber models (Lebreton et al. 1992). In order to minimize the effect of ring-reading errors, we only considered an individual resighted when it was seen at least twice in the same nonbreeding season. In these analyses we only distinguished between juveniles and adults, with immatures considered as adults.

**Depth distribution of the red knots’ wintering food supply (Mauritania)**

Depth distributions for Loripes and Dosinia were taken from the literature (Piersma et al. 1993a), which we corrected for the length distribution that we have found in our benthos samples over the study period 2002-2014 (van Gils et al. 2013), by fitting linear regressions through the published depth-length graphs (Piersma et al. 1993a). The depth distribution of Zostera rhizomes was measured at 8 sites throughout our study area in January 2013, with 4 repeated measures per site at a precision of 1 mm using a ruler.

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Introduction

In a recent article in Current Biology, Streby et al. (2015) reported, that Golden-winged Warblers (Vermivora chrysoptera) performed a facultative migration of >1,500 km southwards to avoid a severe tornadic storm in late April. The storm approached their breeding area in Tennessee, shortly after the proposed arrival of the individuals from their 5,000 km spring migration. The study reports that birds evacuated the breeding area approximately 24 hours before the storm arrived and returned about 5 days later after they lingered in northwest Florida and western Cuba. The authors speculated that infrasound emitted by the storm system could have simulated this evacuation behaviour, noting that infrasound is perceived by birds (Kreithen and Quine 1979) and has been shown to influence their behaviour (Hagstrum 2013). This study purports to show for the first time that small migratory birds are able to perceive severe weather systems and avoid them by long-distance, facultative flight movements away from their breeding grounds and back. It is no surprise that the article received great attention in both the public media and the scientific community. However, there are two points of uncertainty which cause doubts about the validity of these findings. The first is that the method used to track the individual Golden-winged Warblers’ movements is prone to erroneous location estimates, especially with regard to latitude and exact timing. The second is that the extraordinarily long and rapid evacuation flights may not be within the physiological capabilities of Golden-winged Warblers.

These movements were inferred by analysis of data from light-level geolocators deployed on the birds’ backs. These devices record light intensity over time, allowing one to estimate the location of a tag based on geographic variation in the timing of sunrise and sunset. There are a variety of ways to derive
geographic locations from light-level data (Sumner et al. 2009, Pedersen et al. 2011, Lisovski and Hahn 2012) and a full consideration of these tools is beyond the scope of this comment. Streby et al. (2015) used the most frequently applied method (i.e. the simple threshold method), wherein one establishes distinct daily sunrise and sunset times, based on the times at which the light intensity exceeds or falls below a certain threshold. The time period between each consecutive event (length of the day/night) is used to estimate latitude, and the time at which the midpoint between the twilight events (midnight/noon) occurs is used to estimate longitude (Ekström 2004). Estimates of geolocator position are susceptible to any shading that affects the light intensity received by the sensor of the tag (Lisovski et al. 2012). When analysing geolocator data with the threshold method, shading effects on location estimates can be exceptionally pronounced given that each location estimate is calculated independently from previous or subsequent estimates. Importantly, following the spring equinox in the northern hemisphere, shading would tend to shorten estimated daylength and infer a location that is south of the true location. Hence, it is possible that Streby et al. (2015) misinterpreted shade-biased location estimates as evidence of a rapid facultative migration. Shading can be due to many sources (e.g. clouds, smog, microhabitat, topography, and an animal’s behaviour), but the most obvious explanation for shading in this situation is the severe weather system that supposedly triggered the birds’ exodus.

Streby et al. (2015) were aware of the potential shortcomings of light-level geolocation and provided several arguments to support the contention that shading did not influence their location estimates. First, they assert that “transitions (i.e., sunrise and sunset) in the geolocator data were smooth during this period,” which they argued is indicative of shade-free light intensity measures. Streby et al. (2015) also argued that the “study area was not extraordinarily cloudy throughout most of the evacuation period”. Finally they note that the proposed distances travelled in avoiding the storm were substantially greater than the expected error in geolocator location estimates, citing calibration studies documented by Lisovski et al. (2012). However, Lisovski et al. (2012) and other studies (e.g. Fudickar et al. 2012, Liechti et al. 2014, Ross et al. 2014) have shown that the proposed distances between the breeding area and the evacuation locations are within the possible range of error. We thus propose that the interpretation of the geolocator results warrant greater scrutiny.
Furthermore, the physiological feasibility of such rapid and long-distance movements may be challenged. Golden-winged Warblers are nocturnal migrants (del Hoyo et al. 2010). Hence, the birds supposedly perform their evacuation flights during the night. Based on this constraint, some of the daily distances shown in Streby et al. (2015) are extremely long, cf. the “orange bird” in Figure 1 of the original publication. This individual flew about 2,000 km within two nights. Such proposed evacuation movements seem unlikely, especially since most birds were described as having just arrived from their 5,000 km spring migration.

To evaluate our doubts about the conclusions of Streby et al. (2015), we performed a sensitivity analysis to investigate the potential error of estimated locations in the focal region and time. We also re-analysed the raw light-level measurements recorded during the storm event (data available at http://mncoopunit.cfans.umn.edu/home/current-biology-2015-light-level-data/) using the same simple threshold method as described in Streby et al. (2015) and put the corresponding twilight pattern in the context of remotely sensed cloud cover data from birds’ breeding area (MODIS06_L2: ftp://ladsweb.nascom.nasa.gov/allData/51/MOD08_D3). Moreover, we evaluated the proposed movements in terms of potential flight ranges considering 1) regional wind conditions during supposed evacuation flights and 2) general energy requirements.

**Sensitivity analysis**

Our sensitivity analysis was designed to reveal the range of locations that share the twilight times with a given location after accounting for uncertainty. To this end, we define a twilight error model: a probability density function that describes the discrepancy between the expected and the observed twilight times (sunrise/sunset). We used a log-normal distribution to describe this potential discrepancy (Figure A.7.2.1a). The best way to parameterise the error distribution is to use light intensity measurements recorded at a known location and on the focal bird species, and therewith accounting for the naturally occurring amounts of shading. Such measurements are often referred to as calibration data, since it is also used to estimate the correct sun elevation angle for the location estimates of the migratory track, and usually comes from periods just after the deployment of the logger devices.
Unfortunately, calibration data for the Golden-winged Warblers tracked by Streby et al. (2015) were not available from the author. Therefore, as an

Figure A.7.2.1: Kernel density estimates of the error in probable location based on twilight times (sunrise and sunset) weighted against the natural variation in light intensity actually recorded by geolocators deployed on a stationary pole in Switzerland that was unobstructed by vegetation (red), or alternatively on a Common Nightingale (Luscinia megarhynchos) or a Painted Bunting (Passerina crisis) at known locations in France and the United States, respectively. Shown in panel a) is the best fit of a log-normal error probability distribution (black line) based on the pooled geolocator data and defined by the parameters meanlog and sdlog. This probability distribution was used to calibrate the sensitivity analysis in panel b), which illustrates the kernel density estimation of possible positions at which the light intensity pattern from the 28th of April, 2014 could have been recorded, including a black triangle at the threshold-based pinpointed location provided by Streby et al. [1]. The solid and dashed colored lines refer to the 60% and 95% density quantiles from sensitivity analyses using the log-normal parameters from the best fit of each calibration dataset separately (i.e., stationary, nightingale, bunting). The possible locations are based on a Monte Carlo Markov Chain simulation of 2000 sunrise and sunset times. A zenith angle of 93.4° (derived from the log-normal error distribution) was used to estimate locations for the pooled data (93.2° - stationary; 94.3° - nightingale; 93.6° - bunting). The star indicates the breeding area of the Golden-winged Warblers (Vermivora chrysoptera) tracked in Streby et al. 2015.
alternative, we used calibration data from a range of other studies. To show the effect of cloud cover we used long-term dataset from a geolocator deployed on a stick, above the vegetation and with free sight to the sky. Additionally, and due to similar habitat choice as described for Golden-winged Warblers (del Hoyo et al. 2010), we chose calibration data from Common Nightingale (*Luscinia megarhynchos*) (Hahn et al. 2014) and Painted Bunting (*Passerine crisis*). The sensitivity analysis demonstrated that, given the assumed error

![Figure A.7.2.2: a) Deviation in sunrise (solid line) and sunset (broken line) for all five individual Golden-winged Warblers (*Vermivora chrysoptera*) tracked by Streby et al. [1] during the period of the tornadic storm (grey rectangle). Daily deviations in twilight times represent the difference between the estimated sunrise or sunset times from the expected time of civil twilight (sun elevation angle of -6°) at the breeding grounds in eastern Tennessee (84° 17' 38'' W, 36° 16' 8'' N). Panel b) shows the latitude of the estimated locations before and after the tornadic storm event using a zenith angle for each individual which produces the best match to the latitudinal location. Panel c) shows the mean, minimum, and maximum daily values for optical cloud thickness at the breeding area, as remotely sensed by the terra MODIS3 satellite. The same colours were used as in Streby et al. [1] and refer to the same individuals.]

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**Figure A.7.2.2:** a) Deviation in sunrise (solid line) and sunset (broken line) for all five individual Golden-winged Warblers (*Vermivora chrysoptera*) tracked by Streby et al. [1] during the period of the tornadic storm (grey rectangle). Daily deviations in twilight times represent the difference between the estimated sunrise or sunset times from the expected time of civil twilight (sun elevation angle of -6°) at the breeding grounds in eastern Tennessee (84° 17' 38'' W, 36° 16' 8'' N). Panel b) shows the latitude of the estimated locations before and after the tornadic storm event using a zenith angle for each individual which produces the best match to the latitudinal location. Panel c) shows the mean, minimum, and maximum daily values for optical cloud thickness at the breeding area, as remotely sensed by the terra MODIS3 satellite. The same colours were used as in Streby et al. [1] and refer to the same individuals.
distributions in the twilight times (Fig A.7.2.1a), the location estimates as reported for birds by Streby et al. (2015) could have been erroneously inferred from light data recorded at their breeding area in Tennessee, though under average lighting conditions one would not expect such deviations since the breeding area of the Golden-winged Warblers fell outside the 60th percentile of the location density distribution (Figure A.7.2.1b). However, the breeding area is inside the 95th percentile, even when the location distribution was derived from stationary logger calibration, which was influenced only by shading due to weather. In other words, the hypothesis that the warblers simply remained at their breeding grounds cannot be rejected by the data presented by Streby et al. (2015).

**Twilight times and shading during the tornadic storm event**

Assuming that the individuals did not avoid the tornadic storm by flying south, we calculated the discrepancy between the observed and the expected twilight times at the breeding area (Fig A.7.2.2a). For each individual, the expected twilight times were defined as the earliest possible sunrise time or the latest possible sunset time at the breeding site, excluding any shading, as defined by the lowest light intensity the tag is able to detect. This analysis showed that 95% of the twilight time deviations fall within 30 minutes and the maximum deviation was 50.24 minutes (Fig 7.2.2). The actual difference in sunrise and sunset times between the breeding areas in Tennessee and the locations in North Florida at the Gulf of Mexico are 14 minutes. Twilight deviations on this order of magnitude were found in all analysed calibration datasets (Fig A.7.2.1a). Comparing individual deviations around sunrise versus sunset revealed that in most cases the deviations were not similar in their magnitude. This could be due to the individuals moving south-east along a line where sunrise times are similar or, perhaps, south-west where sunset times would be similar. The distance between those locations becomes larger towards the south and, especially in the case of GTN13 (blue), it seems unlikely that the individual switched between the southeast locations with similar sunsets and the southwest locations with similar sunsets within only one day. An alternate explanation for the different deviations would be non-synchronous amounts of shading (cloud-cover) during the sunset versus sunrise twilight periods. While Streby et al. (2015) discount the importance of cloud cover during the storm event, we propose that it could have been a significant factor. Satellite pictures indicating cloud optical thickness, suggest that cloud cover on April 27
to April 30 was higher during the storm event (the period concordant with the tracked birds’ putative evacuation migration) than the days before or after (Fig A.7.2.2c). Furthermore, we propose that the smoothness of the transitions between light and dark provide little information about the role of cloud cover in shading. Because cover cloud cover is quite variable over time, one might indeed predict that the amount of shading through clouds could be evaluated from the “smoothness” of the light-dark transitions. However, the light sensor of the geolocators used by Streby et al. (2015) has a very narrow sensitivity range which detects changes in light within a very small range of very low light intensities. The units are capable of resolving the twilight periods that correspond to a range of sun elevation angles between -5° and -3° below the horizon. Therefore, the time between first detected light and the maximum value being reached would fall between 10 to 14 minutes. Such small time intervals make the light-level profiles uninformative with regards to the degree

![Figure A.7.2.3: Simulated flight trajectories of the individual Golden-winged Warbler (Vermivora chrysoptera) for the proposed evacuation movements on the night from April 27 and April 28. We simulate the movement path using the function NCEP.flight from the R package RNCEP (Kemp et al. 2012). We defined bird’s airspeed to be 10 m/s. Based on this assumption and by utilizing the wind conditions at one of four altitudes (i.e., pressure levels: 1000, 925, 850, and 700 mbar) which was most favourable to flying to Steby et al.’s (2015) next putative stopover location, we estimated each individual’s nightly flight range from local dusk at the starting locations to local dawn at the destination (plus one hour). The feasible part of the path is given by the unbroken coloured line, whereas the unfeasible part of the path is indicated by the broken black line. Colors refer to the same individuals as in Streby et al. (2015). Evaluation for the other dates is given in document S3. Grey arrows indicate wind direction and relative velocity at a pressure level of 1000 mbar, the level where the majority of birds experienced the most favourable wind towards the proposed location. The lengths of the arrows are scaled to reflect wind velocity across the geographic region.](image)
to which cloud cover or location-specific effects produced differences in the smoothness of light increase or decrease. Regardless of the amount of shading, transitions will always appear rapid and smooth, even though they may be temporally-shifted (i.e., later at sunrise, earlier at sunset).

**Movement ecology**

To evaluate the feasibility of the proposed evacuation flight, we used the *NCEP* simulation function from the R package *NCEP* (Kemp et al. 2012), which simulates movement trajectories based on defined speed capacities and incorporates wind data at given pressure levels. Streby et al. (2015) provided just one location per day, which correspond to consecutive sunrise and sunset times that were experienced at roughly the same location. From this information we infer that actual movements occurred at night, which is typical of nocturnal migrants like Golden-winged Warblers. We defined the birds’ air speed to be 10 m/s, which is slightly higher than the air speed of similarly-sized *Sylvia* warblers in Europe (Bruderer and Boldt 2001). Birds were allowed to choose the most favourable wind conditions out of four pressure levels (1000, 925, 850, or 700 millibars) to reach the next corresponding location. These pressure levels correspond to an altitude range of 0 - 4,000 meters above sea level. Birds were allowed to start their nightly flight at sunset and until 1 hour after sunrise.

The resulting flight ranges were compared with the shortest distance, i.e., great circle distance, between the location estimates for each individual as given in Streby et al. (2015). In nine out of 23 movements the birds reached the proposed location during the simulation. The remaining 14 flights stopped an average of 312 ± 201 km (mean ± sd) short of the proposed location. Wind conditions especially in the night of the 27th and 28th of April, the nights the individuals were proposed to have abandoned the breeding area and flown southward, were extremely unfavourable for such movements (Fig A.7.2.3).

**Energy requirements**

Considering the proposed evacuation movements, Golden-winged Warblers had to prepare daily for their next flight by rapidly replenishing the fuel used during the previous flight. To model the energy requirements for the proposed flights in terms of changes in body mass, we assumed that all individuals had a body mass of 10 g (Peterson et al. 2015, Streby et al. 2015). Based on the individually wind-mediated flight durations to actually reach their next location.
estimate (see NCEP-flight simulation above), we let birds “fly” until they had reached their next location estimate while losing 1% of their current body mass every hour of their flight (Delingat et al. 2008). Birds were allowed to replenish fuel by a high hourly fuel deposition rate of 0.005 relative to their lean body mass of 8 g (cf. Dierschke et al. 2005). On an average 14-h day a Golden-winged Warbler would put on 14 [hour] x 0.005 [1/hour] x 8 [g] = 0.56 g of fuel. If the individuals required more than the time period between sunset and sunrise for flying (Fig A.7.2.3), they were only allowed to refuel for the remaining daylight period before the start of the next flight. The current evening body mass was used as the starting body mass for the next flight. Results of this simplified model demonstrated that three out of the five Golden-winged Warblers would have returned to their breeding area in body conditions far below expected lower limits (<7.2 g) (Fig A.7.2.4).

**Conclusions**

Cause for doubt concerning the interpretation of the geolocation data by Streby et al. (2015) can be found in our sensitivity analysis (Figure A.7.2.1), the
asynchronous change in sunset versus sunrise twilights (Figure A.7.2.2), and the unfeasibility of the proposed flight ranges (Figures A.7.2.3-4). An alternative explanation for the observed light-level data is that the Golden-winged Warblers did not avoid the approaching tornadic storm by lengthy evacuation movements, but stayed within the proximity of the breeding area. The shifts in twilight times can be explained by normal environmental shading, let alone the passing of a severe thunderstorm front and its inevitable shading and/or associated sheltering behaviours exhibited by birds.

It is also possible that some individuals did not actually arrive at the breeding grounds prior to the storm system and were thus unable to perceive the weather system while at the breeding site. Streby et al. (2015) state that 3 of the 5 birds they tracked arrived only on April 26-27 and that these then left the area just ahead of the storm on April 28. For reasons discussed above and elsewhere solar geolocation data are poorly suited for establishing brief visits to specific locations. Moreover, simulations aside, we question the capacity of these birds to move as described. Consider that GTN09 (green), which reportedly arrived at the breeding area on the 27th of April only to perform a >500 km flight southward the following night. This despite wind conditions on the April 27 and April 28 being unfavourable for southward movements (Fig A.7.2.3).

We do not dispute the notion that some version of the phenomenon described by Streby et al. (2015) is possible. Indeed, there are instances of migration from a breeding site in response to harsh weather conditions [Rakhimberdiev pers. obs.] and avoidance of bad weather is known to occur during migration (see “reverse migration”: Richardson 1978). However, there are many documented cases where, in the aftermath of violent storms such as those that struck Tennessee in 2014, multitudes of avian victims of the violent storms are found (McClure 1945; Ross pers. obs., Smith and Webster 1955). Birds appear to take shelter from impending storms, notably subduing or ceasing singing behaviors as early as 48-hours prior to the storm (Linney 1898). Indeed at other locations in the southern Appalachian region, Golden-winged Warblers continued to be seen during the period of the April 27-30 evacuation migration (http://ebird.org/).

Arguably, Streby et al. (2015) have proposed an intriguing phenomenon that should motivate researchers to investigate the behavioral response of particularly mobile species such as birds to severe weather events. Yet, we
again stress that light-level geolocators are limited in their ability to investigate such potentially short-term and small-scale movements. Analysing light-level geolocation data is complex and requires that one first considers whether the derived location estimates are generally appropriate and, second, whether the applied evaluation process provides the required spatiotemporal resolution to test the initially formulated hypothesis. Science’s fundamental principles of objectivity, reproducibility, and transparency are not often guaranteed in light-level geolocation studies. To this end we call for a set of guidelines that establish best-practices for the analysis of geolocator data, and we call upon journal editors to require data sharing, including the fundamental calibration data, via an online archive such as Movebank (www.movebank.org). Such requirements were established long ago for genetic data and research in the associated fields has thrived as a result (cf. Merrill and Mazza 2006). Only when in-depth details about the analyses steps, ideally the code, and the raw data are made available will light-level geolocation serve as a stronger and more objective tool for movement ecology.

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