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Capturing individual-level parameters of influenza A virus dynamics in wild ducks using multistate models

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Summary

1. Disease prevalence in wildlife is governed by epidemiological parameters (infection and recovery rates) and response to infection, both of which vary within and among individual hosts. Studies quantifying these individual-scale parameters and documenting their source of variation in wild hosts are fundamental for predicting disease dynamics. Such studies do not exist for the influenza A virus (IAV), despite its strong impact on the global economy and public health.

2. Using capture–recaptures of 3500 individual mallards *Anas platyrhynchos* during seven migration seasons at a stopover site in southern Sweden, we provide the first empirical description of the individual-based mechanisms of IAV dynamics in a wild reservoir host.

3. For most years, prevalence and risk of IAV infection peaked at a single time during the autumn migration season, but the timing, shape and intensity of the infection curve showed strong annual heterogeneity. In contrast, the seasonal pattern of recovery rate only varied in intensity across years.

4. Adults and juveniles displayed similar seasonal patterns of infection and recovery each year. However, compared to adults, juveniles experienced twice the risk of becoming infected, whereas recovery rates were similar across age categories. Finally, we did not find evidence that infection influenced the timing of emigration.

5. *Synthesis and applications*. Our study provides robust empirical estimates of epidemiological parameters for predicting influenza A virus (IAV) dynamics. However, the strong annual variation in infection curves makes forecasting difficult. Prevalence data can provide reliable surveillance indicators as long as they catch the variation in infection risk. However, individual-based monitoring of infection is required to verify this assumption in areas where surveillance occurs. In this context, monitoring of captive sentinel birds kept in close contact with wild birds is useful. The fact that infection does not impact the timing of migration underpins the potential for mallards to spread viruses rapidly over large geographical scales. Hence, we strongly encourage IAV surveillance with a multistate capture–recapture approach along the entire migratory flyway of mallards.

Key-words: avian influenza, epidemiology, host–pathogen dynamics, individual-based monitoring, influenza A virus, multistate capture–recapture, outbreaks, SIR model, waterfowl, zoonosis

Introduction

Sixty per cent of emerging infectious diseases originate from wildlife (Jones *et al.* 2008). Hence, a complete understanding of the mechanisms underlying infectious disease dynamics and emergence in wild population is fundamental to public health, wildlife conservation and socio-economics. The dynamics of diseases are essentially driven by changes in the rates at which susceptible host individuals become infected by a pathogen (force of

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infection) and clear this infection (recovery; McCallum, Barlow & Hone 2001). These individual-based epidemiological parameters are, in turn, affected by host life history (Hawley & Altizer 2011), as well as changes in host behaviour (e.g. sociality, migration), physiology and/or immunology (susceptibility, resistance) in response to infection (Beldomenico & Begon 2010). Therefore, integrative empirical studies linking individual changes in epidemiological parameters and response to infection to the temporal variation in pathogen prevalence are the bedrock for predicting and understanding wildlife disease dynamics (Altizer *et al.* 2006). Despite this, such work remains extremely rare (Tompkins *et al.* 2011). This is particularly the case for important wildlife diseases with severe impacts on humans, such as the zoonotic viruses: rabies, ebola and avian influenza (IAV).

One of the major reasons for this lack of documentation is due to the costs associated with maintaining longitudinal monitoring of infections in free-ranging individuals, as well as the challenge in obtaining reliable empirical epidemiological parameters and assessing their sources of variation (e.g. for IAV Hoye *et al.* (2010)). Research focusing on disease dynamics is often limited to data on population prevalence rates. Because a given prevalence variation pattern can result from variation within and between an individual in (i) infection parameters only, (ii) recovery parameters only, or (iii) both parameters, prevalence records are not enough to assess the mechanisms underlying disease dynamics. When estimations of infection and recovery parameters are produced, they often arise from compartmental models fitted to prevalence data [e.g. IAV in waterfowl (Brown *et al.* 2013; Hénaux *et al.* 2013), cowpox and tuberculosis in voles *Microtus agrestis* (Cavanagh *et al.* 2004), and rabies in bats *Eptesicus fuscus* (George *et al.* 2011)]. Such estimations strongly depend on the underlying model assumptions, which set the sources of variation for the different epidemiological parameters.

Intensive monitoring of free-ranging hosts, with systematic capture–recaptures of individuals and infection diagnosis, remains the gold standard for deriving information on pathogen dynamics in the wild. Such sampling, in concert with multistate capture–recapture (MS-CR) modelling, provides an efficient method for identifying the individual-based mechanisms of disease dynamics in wild systems. Examples include cowpox virus in bank voles *Clethrionomys glareolus* and wood mice *Apodemus sylvaticus* (Begon *et al.* 1999), and tuberculosis in European badgers *Meles meles* (Graham *et al.* 2013). MS-CR models were originally developed for estimating demographic parameters (survival, breeding, migration) based on longitudinal monitoring of free-ranging individually marked animals (Lebreton & Pradel 2002). By accounting for heterogeneity in individual detectability due to varying capture rates or permanent emigration, these models allow unbiased empirical estimates of individual-based transition rates between different biological states (e.g. resident/emigrant; infected/non-infected). As a result, sources of variation in transition rates and the effects of individual states on various demographic parameters can be investigated. This makes MS-CR models ideal for studying the individual-based mechanisms of pathogen dynamics and the consequences of infection on host demography [reviewed in Cooch *et al.* (2012)].

Here, we use this framework to provide the first empirical investigation of the individual-based mechanisms underlying IAV dynamics in a wild reservoir host. Although IAV has a broad host range, dabbling ducks of the genus *Anas* are the most important natural reservoir (Webster *et al.* 1992; Olsen *et al.* 2006). These birds are considered to be the major source of all low pathogenic IAVs (LPIAVs), some of which can mutate into highly pathogenic strains (HPIAVs) when spilling over to domestic poultry (Munster *et al.* 2007). HPIAVs have been responsible for severe outbreaks in domestic and wild birds and have zoonotic potential, causing infections and deaths in humans (Webster *et al.* 1992; Stallknecht, Brown & Swayne 2008). Given that wild birds serve as the predominant hosts for IAVs, disease surveillance in wild populations has been conducted globally. The longest and most intense studies come from Europe (Munster *et al.* 2007; Latorre-Margalef *et al.* 2014) and North America (Krauss *et al.* 2004; Wilcox *et al.* 2011). Such studies have provided a good description of spatiotemporal variation in IAV prevalence in wild ducks, especially within and among mallard *Anas platyrhynchos* populations. The annual IAV disease pattern in mallards appears to be consistent across years and regions of the Northern Hemisphere: high prevalence during autumn at the time of post-breeding aggregation and migration, dropping off as winter starts, and being generally low during spring migration and the breeding period (Munster *et al.* 2007; Wilcox *et al.* 2011). Additionally, prevalence in first-year birds is usually higher than in adults (Wallensten *et al.* 2007), which is attributed to the immunologically naïve status of young birds. This has also been shown in experimental studies (Costa *et al.* 2010). However, despite these studies and the global impact of IAV world-wide, individual-based mechanisms underlying IAV disease dynamics have not been unravelled (Hoye *et al.* 2010).

Based on MS-CR modelling and a unique data set of ~3500 capture–recapture histories of mallards tested for IAV during seven migration seasons in southern Sweden, we aimed to (i) provide the first empirical estimates of the epidemiological parameters driving IAV dynamics in the wild reservoir (i.e. individual infection and recovery rates, Fig. 1), (ii) describe their sources of variation due to the effects of season, year and age (Table 1), (iii) assess individual changes in response to infection, such as individual immunity development (translated into changes in recovery rates) or alteration of migratory behaviour, and (iv) link these individual changes to the observed population prevalence patterns.

Materials and methods

STUDY SITE, SPECIES AND IAV SCREENING

The study site was located in Ottenby, the southernmost tip of Öland island in the Baltic Sea (56°12′N 16°24′E). At this site, wild mallards were captured daily from April to December each year in a duck trap where a few sentinel ducks were also kept captive (for details see Latorre-Margalef *et al.* (2014)). Each wild captured duck was individually identified, aged (juvenile, adult or unaged), sexed according to plumage criteria, measured (wing, tarsus and head (bill-tip to the back of the skull) lengths in millimetres and mass in grams) and sampled for IAV before being released back to the wild. Individuals were regularly recaptured over the course of their stopover at Ottenby during autumn migration (August–December). For each capture/recapture, birds were sampled for IAV. Up to three different types of samples were taken for IAV detection from each individual: cloacal swabs (by swirling a sterile swab in the cloaca), oropharyngeal swabs (by swabbing the lower part of the oropharyngeal tract) and fresh faeces (from the bottom of single-use cardboard boxes if ducks defecated in them). Samples were stored in virus transport media at −70 °C until they were analysed in the laboratory, and the infection status of individuals at each capture/recapture was assessed by real-time PCR amplification of the IAV matrix (see Latorre-Margalef *et al.* (2014)). PCR-positive samples were propagated in pathogen-free embryonated chicken eggs, and the resultant viruses were subtyped.

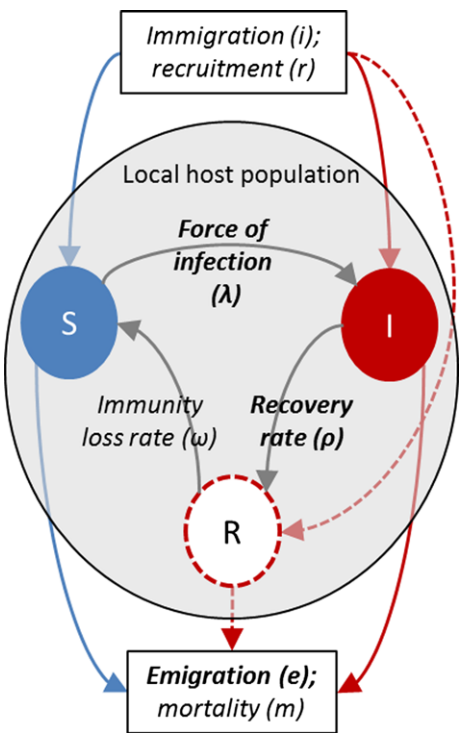

Fig. 1. Conceptual model for the mechanisms underlying local dynamics of influenza A viruses in our mallard population. Epidemiological and demographic parameters investigated in the present study are shown in bold. 'S': Susceptible hosts; 'I': infected hosts; 'R' recovered hosts.

CAPTURE–RECAPTURE HISTORIES

For each daily inspection of the trap, hereafter called capture occasion, the fate of each individual bird was classified into three observations: '1' captured and negative for IAV, '2' captured and positive for IAV and '0' not captured (for situations where individuals had not yet been captured for the first time or had been previously captured at the trap but were not recaptured on that occasion). Sorted chronologically for each individual, these observations resulted in individual MS-CR histories which took the form of a sequence of 0s, 1s and 2s. For example, sequence '00012002', described an individual captured for the first time at the fourth capture occasion and not infected, then recaptured and infected at the fifth and eighth capture occasions.

Over 7 years (2002–2008), from 1 August to 16 December, a total of 5349 MS-CR histories could be generated (Table S1, Supporting information). One assumption of the MS-CR models (which are described below) is that the capture occasions occur over short periods of time compared with the intervals between successive capture occasions (Nichols 1992). To remedy this, only information collected every second day was considered, leading to 70 capture occasions each year. This resulted in 3539 two-day individual MS-CR histories comprising 5490 captures/recaptures of birds negative for IAV and 919 for birds positive for IAV, of which 365 were characterized to subtype level. Mallards use our study site during migration stopover for only a few days or weeks in any given year. As a result, the MS-CR histories were sparse (Table 2).

MULTISTATE CAPTURE–RECAPTURE MODELS

We used MS-CR models (Lebreton & Pradel 2002) implemented in the program E-SURGE (Choquet, Rouan & Pradel 2009), for estimating individual epidemiological transition parameters from the MS-CR histories. MS-CR models such as the Arnason–Schwarz model ('AS' see Arnason (1973) and Schwarz, Schweigert & Arnason (1993)) are extensions of the Cormack–Jolly–Seber (CJS) model (Cormack 1964; Jolly 1965; Seber 1965), which was designed to simultaneously estimate mortality and recapture probabilities from incomplete CR histories of marked individuals. The AS model generalizes the CJS model to situations where CR histories include information on non-permanent individual characteristics such as epidemiological states (e.g. Faustino *et al.* (2004); Senar & Conroy (2004)). Here, we used AS model including parameters for recapture probability (P), apparent mortality between consecutive occasions (e), transition probabilities from non-infected to infected state between consecutive occasions (λ, i.e. the infection force in Fig. 1) and transition probabilities from infected to non-infected state between two successive occasions (ρ, i.e. the recovery rate in Fig. 1).

The AS model assumes that whenever an individual is captured, its state is known with certainty. As a consequence, we assumed that IAV infection status of captured mallards was known without error (i.e. perfect sensitivity and specificity of the IAV test). RT-PCR assays generally have high specificity, whereas sensitivity can be an issue (Lemmon & Gardner 2008). If this is the case for the standard IAV test, then the epidemiological parameters derived in our study might be slightly underestimated. Furthermore, true mortality and permanent emigration cannot be distinguished in our data as both result in the permanent disappearance of an individual from the study area.

Table 1. Source of variation considered for each focal parameter and its biological significance

	Emigration	Recapture	Infection/recovery
Age	Varying stopover duration among age classes	Trap attractiveness depending on experience/age	Different immune protection between age classes
Infection state (IS)	Infection modifying migratory behaviour	Infection modifying behaviour	
Seasonal trend (S)	Seasonal variation in migration phenology	Crowding and social learning during the season	Varying prevalence and consecutive infections along the season affecting host susceptibility
Year (Y)	Varying stopover duration among years due to climatic conditions	Yearly heterogeneity due to climatic conditions	Varying virus populations and host local density among years affecting transmission risks
Age × Seasonal trend (Age × S)	Varying migration phenology depending on age		Seasonal patterns varying between age classes (e.g. delayed infections in adults)
Year × Seasonal trend (Y × S)	Varying migration phenology due to climatic conditions		Seasonal patterns varying among years due to circulating subtypes, arrival of birds
Transience (Tr)	Varying stopover duration among individuals		
Trap dependency (Td)		Birds avoiding or returning to the trap due to the presence of food	

Table 2. Number of recaptures for each individual multistate capture–recapture (MS-CR) history

Number of recaptures	0	1	2–4	5–10	11–20
Number of MS-CR histories	2114	562	403	131	29

However, given that consecutive occasions were separated by only 2 days, true mortality was negligible as compared to emigration and apparent mortality was thus interpreted as emigration probability (and therefore denoted e). In AS models, the different parameters are allowed to vary over time, between states or according to individual characteristics (such as age and sex). Specifically, parameters were allowed to vary according to age, year and smooth functions (i.e. fourth-order polynomial) of date. In addition, emigration probability between occasions t and $t + 1$ was allowed to vary according to the epidemiological state at occasion t, whereas recapture probability at occasion $t + 1$ depended on the epidemiological state at $t + 1$ (Fig. S1). Because the AS model assumes first-order Markovian transitions, longer lasting effects of epidemiological states could not be considered.

GOODNESS-OF-FIT TESTS

Specific goodness-of-fit tests are lacking for AS models. Instead, we relied on *ad hoc* pooling tests proposed for the Jolly-Move multistate model, which is a generalization of the AS model (Table S2). Short- and long-term trap dependencies (capture of an individual at a given occasion affects its subsequent risk of recapture in the near future) as well as transience (strong interindividual heterogeneity in emigration probability) were detected. These departures from AS model assumptions were accommodated by (i) including transient effects by allowing emigration over the time interval following the first capture to differ from emigration over the subsequent time interval, and (ii) a three-class trap-dependence effect on the capture parameter, following previously suggested approaches (Pradel 1993; Pradel *et al.* 1997). The three-class trap-dependence effect allowed recapture probability at t to depend on whether the last capture of the individual has occurred at $t-1$, $t-2$ or $t-3$.

MODEL SELECTION STRATEGY

Statistical models explaining variation in each parameter were identified following a three-step approach. In step 1, we constructed a model including all explanatory variables considered to be potentially important for each focal parameter. This starting model did not include any interactions and is referred to as a full additive model. We then explored the neighbourhood of this model by removing each main effect one at a time and by adding each potentially important two-way interaction one at a time. This step allowed us to identify the main significant effects and interactions using likelihood ratio tests (LRTs; Table S3). In step 2, a consensus model including important interactions, and excluding irrelevant effects according to the LRTs in step 1, was fitted to the data (Table S4). Finally, in step 3, the neighbourhood of the consensus model was explored using LRTs (Table S4). Additionally, Akaike Information Criterion was computed for each model (Burnham & Anderson 2002).

INTERANNUAL AND SEASONAL VARIATION IN PREVALENCE

The number of positive IAV samples and the total number of samples were computed for each age category, each week of the

study period. Seasonal patterns of IAV prevalence were then depicted for each year and each age class independently by fitting distinct general additive models (GAMs) that included spline functions of week on the binomial prevalence response variable. GAMs were fitted with the mgcv package in the R software (R Development Core Team 2007).

Results

POPULATION-SCALE VARIATION IN IAV PREVALENCE

Influenza A virus prevalence fluctuated at around 20% in juveniles and 10% in adults (Fig. 2). The seasonal pattern of prevalence varied by year, but in a similar way in juveniles and adults. In all years except 2006, prevalence peaked from early October until mid-November and then dropped markedly in December.

INDIVIDUAL-SCALE VARIATION IN INFECTION FORCE AND RECOVERY RATE

The model selected for the infection force included effects of age, year, season and the interaction between year and season (model $\lambda_{Age + S + Y \times S}$, Table S4). The estimated infection force showed yearly seasonal variations (LRT for interaction $Y \times S$: $\chi^2 = 63{\cdot}1$, d.f. = 24, $P = 0{\cdot}00002$). The individual infection force was generally high from early October until mid-November and then dropped markedly in December. In some years, estimated infection force showed either an additional early peak (2003, 2005), or little variation over the season (2004, 2006). The year 2002 stood out with a late peak (Fig. 2).

Each year, the infection force was higher in juveniles than in adults (odds ratio [OR] $\approx 2{\cdot}18$) and intermediate in unaged individuals (LRT for *Age* effect: $\chi^2 = 7{\cdot}9$, d.f. = 2, $P = 0{\cdot}02$). This between-age difference in infection force remained constant during the season (LRT for interaction $Age \times S$: $\chi^2 = 13{\cdot}0$, d.f. = 8, $P = 0{\cdot}11$). In most years, the infection force, estimated for a two-day period, varied from 0 to 0·10 in juveniles and 0 to 0·05 in adults. Maximum estimated two-day infection force was around 0·30 in juveniles and 0·20 in adults.

For recovery rate, only season and year effects were retained in the final model (model $\rho_{S + Y}$; Table S4). Recovery rates varied over the autumn migratory season (LRT for *S*: $\chi^2 = 13{\cdot}3$, d.f. = 4, $P = 0{\cdot}01$), but with a pattern that was consistent across years (LRT for $Y \times S$: $\chi^2 = 24{\cdot}5$, d.f. = 24, $P = 0{\cdot}43$) and among age categories (LRT for $Age \times S$: $\chi^2 = 11{\cdot}9$, d.f. = 8, $P = 0{\cdot}16$). For any year and age category, estimated recovery rates were high at the beginning of the season in August, decreased until mid-September, remained low until the end of October and then slowly increased to reach high values again in December (Fig. 2). Recovery rates also varied among years (LRT for *Y*: $\chi^2 = 35{\cdot}2$, d.f. = 6, $P < 0{\cdot}0001$); being relatively high in 2003, 2004 and 2006 and relatively low in 2002, 2005, 2007 and 2008; but not with the age of birds (LRT for *Age*: $\chi^2 = 0{\cdot}8$, d.f. = 2, $P = 0{\cdot}67$). Overall, two-day recovery rates were always higher than 30%, which corresponds to a virus shedding period ranging from 1·0 to 6·8 days.

VARIATIONS IN EMIGRATION AND RECAPTURE PROBABILITIES

With respect to sources of variation in emigration probabilities, we failed to detect any influence of infection (LRT for *IS*: $\chi^2 = 0{\cdot}7$, d.f. = 1, $P = 0{\cdot}42$). Only effects of age, transience, season and year, as well as interactions between year and season, age and season, and transience and season, were retained (final model: $e_{Age + Tr + S + Y + Y \times S + Age \times S + Tr \times S}$). Emigration probabilities varied over the season, and the seasonal pattern differed among years (LRT for interaction $Y \times S$: $\chi^2 = 85{\cdot}2$, d.f. = 24, $P < 0{\cdot}0001$). Years 2002–2005 were characterized by similar emigration patterns peaking in mid-season, from mid-September to mid-October (Fig. S2). In contrast, 2007 and 2008 showed two distinct emigration peaks, one early (September), the other towards the end of the season (November). Year 2006 stood apart, with emigration rates being almost constant across the season. The seasonal pattern of emigration also varied among age categories (LRT for interaction $Age \times S$: $\chi^2 = 34{\cdot}1$, d.f. = 8, $P < 0{\cdot}0001$). Finally, we also found a significant interaction between transience and season (LRT for interaction $Tr \times S$: $\chi^2 = 15{\cdot}0$, d.f. = 4, $P = 0{\cdot}005$), which suggests that the proportion of transient individuals (i.e. individuals settling in the study site for <2 days) varied along the season.

With respect to variation in recapture probabilities, the selected model included effects of infection state, age, trap dependence, season, year and interaction between year and season (model $p_{IS + Age + Td + S + Y + Y \times S}$, Table S4). All these effects were highly significant (all *P*-values <0·005). In all years, the seasonal variation in recapture probability showed a single peak, early in the season for years 2004 and 2007, in mid-September for year 2003, or in late October to mid-November for years 2002, 2005, 2006 and 2009. These patterns mirrored the seasonal variation in the number of birds caught (Figs S3 and S4). In addition, recapture probabilities varied among age categories (LRT for *Age*: $\chi^2 = 23{\cdot}2$, d.f. = 2, $P < 0{\cdot}0001$), but the seasonal pattern was identical for each age category (LRT for $Age \times S$: $\chi^2 = 9{\cdot}6$, d.f. = 8, $P = 0{\cdot}29$). Recapture probabilities were higher for juveniles than adults (OR $\approx 1{\cdot}6$) and intermediate in unaged individuals. Recapture probabilities were also higher for infected than non-infected birds (OR $\approx 2{\cdot}8$; LRT for *IS*: $\chi^2 = 8{\cdot}4$, d.f. = 1, $P = 0{\cdot}004$). Overall, recapture probability estimates depended on date, age and infection, ranging from <0·05 for uninfected adults to around 0·80 for infected juveniles.

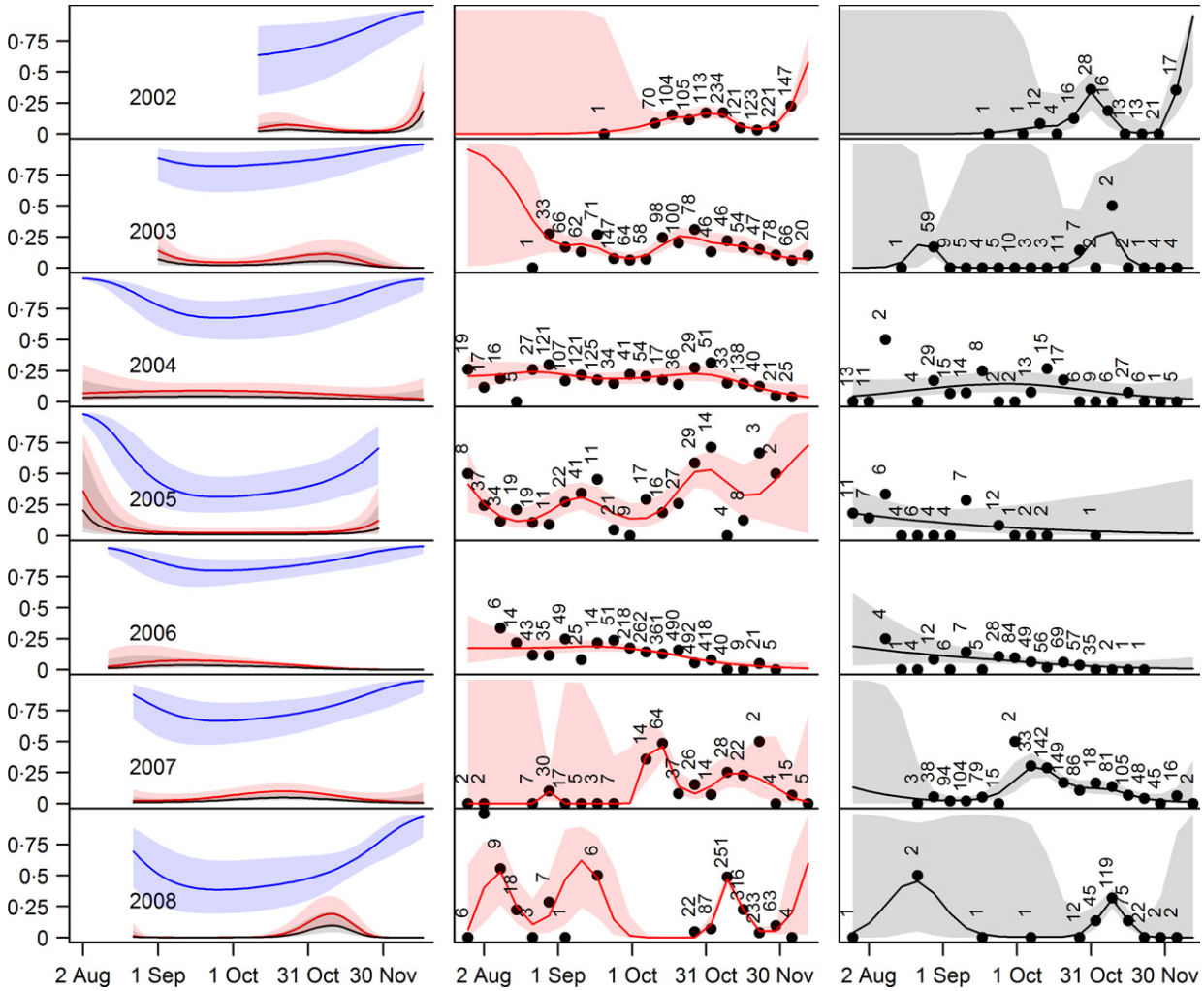

Fig. 2. Seasonal variation in individual-based epidemiological parameters and prevalence across years. Left panels: infection force (red: juveniles; black: adults) and recovery rates (blue). Estimates (lines) and 95% CI (shading) are derived from the final model. Middle panels: prevalence estimates in juveniles. Right panels: prevalence estimates in adults. Prevalence estimates (lines) and 95% CI (shading) are derived from generalized additive models. Black dots: observed prevalence; numbers: sample size.

Discussion

The dynamics of infectious diseases in wild populations is mainly governed by individual changes in infection and recovery parameters. Assessing their sources of variation in wildlife is urgently needed for predicting and controlling the spread of infectious diseases. Though challenging, this information can be obtained through individual-scale monitoring of infectious states in wild populations and MS-CR modelling (Cooch *et al.* 2012). We used this approach to unravel the essential components of IAV epidemiology in wild individual mallards. To our knowledge, this is the first time infection and recovery parameters of IAV dynamics have been estimated at the individual level in the wild reservoir. Additionally, this is the first empirical assessment of the main sources of variation in IAV epidemiological parameters.

We found strong interannual variation in the seasonal patterns of infection risk in mallards. Despite this, some patterns were recurring each year. First, juveniles experienced nearly twice the risk of becoming infected by IAV than adults. Secondly, in all years (except 2005) the risk of infection in both age categories peaked at a single time in September, October or November. Generally, the timing of the seasonal peaks of infection risk and prevalence coincided. Previous studies have reported IAV prevalence peaks in mallards during autumn migration in the Northern hemisphere (Wilcox *et al.* 2011; van Dijk *et al.* 2014; Latorre-Margalef *et al.* 2014) and have mainly attributed these patterns to migration phenology. In autumn, mallards utilizing our study site are migrating from breeding areas in the Baltic states and Russia, towards wintering areas in north-west Europe, for example Denmark, Germany, the Netherlands and France (Gunnarsson *et al.* 2012). To some extent, the seasonal variation in recaptured birds in our population reflects local changes in population size following arrival and departure of migrants (Figs S3 and S4). Birds breeding in closer areas

(Baltic Sea area) arrive first in the season, followed by those breeding further to the east in Russia (Gunnarsson *et al*. 2012). By mid-season (~October), birds from most origins are staging at our stopover site, resulting in a temporary growth in population size. At this time, high contact rates due to congregation of birds, as well as inputs of viruses from different populations, likely increase the risk of infection. In years 2002, 2004, 2007 and 2008, the seasonal peak of infection coincided with the seasonal peak of birds recaptured in the trap. This confirms that variation in the timing of immigration at the stopover site, likely in relation to annual weather conditions, is an important driver of the variation in the seasonal peak of infection and IAV prevalence observed each year. However, for other years the absence of clear correspondence between the seasonal risk of infection and trends in birds recaptured at the stopover site suggests other mechanisms also underpin the epidemiological patterns.

Besides changes in host population size, changes in host response to infections during the season might affect the shape of the epidemiological pattern, that is the intensity and duration of the epidemics. Primary infections in mallards have been shown to confer a partial immunity against subsequent infections by the same virus subtype (homo-subtypic immunity) or by different subtypes (hetero-subtypic immunity), resulting in a shorter shedding period and fewer viral particles excreted in reinfections (Jourdain *et al*. 2010; Latorre-Margalef *et al*. 2013). A lower risk of infection in adults compared to juveniles over the season was likely related to immunological memory afforded by antibodies, which naïve juvenile birds had not yet developed (Tolf *et al*. 2013). Similarly, the reducing risk of infection late in the season may result from individual immunity development throughout the season, which is supported by the seasonal variation in recovery rates. Each year in August, before migration started in earnest, birds had recovery rates close to one, that is a shedding duration <2 days. Later, around mid-September, recovery rates dropped significantly, sometimes below 0·40, that is a shedding duration of around 5 days. This decline was always followed by a slow increase back to original recovery rates towards the end of the season. This increase in recovery rates in the second half of the migration season is consistent with host immunity development. At this time, most birds in the population would have been infected at least once and acquired protection against the circulating subtypes, which translates into reduced duration of shedding (Costa *et al*. 2010). However, the mechanisms behind the sudden drop in recovery rates observed in the early season (August–September) are less obvious. In our population, the subtype composition is known to vary between years but also within a season, in response to annual variations in migration phenology (Latorre-Margalef *et al*. 2014). At the beginning of the season, birds from various origins bring new viruses to the population resulting in an increase in local subtype diversity. As such, the annual drop in recovery rates and the intensity of this drop are likely attributed to (i) lack of protection of the local hosts against the pool of subtypes introduced by immigrants, and (ii) lack of protection of the immigrants against the local subtypes. The absence of age difference in the pattern of recovery rates supports this scenario, since both adults and juveniles would be naïve regarding these novel viruses.

Finally, while arrival of birds, subtype diversity and individual immunity development appear to be important sources of variation in the epidemiological patterns observed at the stopover site, changes in host behaviour due to infection seem to play a minor role. Birds rely on fat storage before departing to provide sustained energy throughout flight (Newton 2008). In our population, infection in wild mallards is associated with a slightly lower body mass (2%) compared to non-infected individuals (Latorre-Margalef *et al*. 2009). Hence, we expected infected birds to prolong stopover because of lower rates of replenishment compared to non-infected individuals and that this would in turn affect the duration of epidemics. However, we found no evidence that infection affected the timing of departures (Fig. S2) or that infection altered mallard movement activity at our study site (Bengtsson *et al*. 2016).

In contrast, we detected a substantial effect of infection on recapture rates, whereby infected birds had a higher chance of being recaptured than non-infected birds. Grain is provided in the trap, and infected birds, experiencing a slight loss in body mass, could adopt a cheap foraging behaviour by revisiting the trap. When caught in the trap, birds share the same water pool for a couple of hours until release, which might favour virus transmission between individuals at that time. Combined with the strong tendency of birds to return to the trap, this would explain the higher recapture rates of infected individuals. Under this scenario, one may question the relevance of the infection patterns described here and their repeatability in natural conditions. We believe that our results reflect the natural transmission of the virus between hosts since the trap mimics the small pond habitat that wild mallards favour, and share with aggregated conspecifics for days to weeks during stopover. In other words, the epidemiological mechanisms captured in our study would at most amplify, but not distort natural processes.

Beyond providing important insights into our understanding of disease dynamics in wild populations, we believe that our results will help to improve IAV surveillance. In our population, the seasonal dynamics of IAV was subject to strong interannual variation rendering forecasting difficult. However, we found that the peak of prevalence was related to the timing and intensity of the peak of infection probability. In this context, prevalence records can be used for risk assessment along the migratory flyway of mallards since they reflect the seasonal risk of infection. However, parts of the epidemiological processes observed in our study were driven by the

seasonality of autumn migration in temperate regions. Differences are expected in breeding grounds but also in sites where communities of birds aggregate all year round, such as in tropical regions (Gaidet *et al.* 2011). Since epidemiological parameters are needed to assess the reliability of prevalence patterns as a proxy for the risk of infection, we strongly encourage individual-based surveillance at these sites. In this context, captive sentinel birds kept in close contact with wild birds can limit the cost associated with capture–recaptures of wild animals. For example, in our population, most of the patterns depicted in wild birds were also observed in the sentinels kept in the trap (results not shown). Furthermore, we found that IAV infections in mallards do not delay migration. This result highlights the potential for mallards to spread the virus rapidly during migration. Hence, we strongly recommend IAV surveillance at the main aggregation sites along the migratory flyways of mallards. Finally, since LPIAVs and HPIAVs, such as H5N1 and H5N8, have similar characteristics (duration, symptoms) when infecting the natural reservoir (Pantin-Jackwood *et al.* 2015), we believe that the results and conclusions presented in this work can be extended to HPIAVs and thus help to predict outbreaks.

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Author contributions

AA and VG wrote the manuscript and ran the statistical analyses, NLM and CT performed fieldwork and molecular analyses, BO and JW provided the data, conceived, designed and run the experiment, and JW, NLM, CT, NG and BO provided substantial inputs and revisions of earlier versions of the manuscript.

Data accessibility

Data are available in Appendix S1.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Fate transition diagram from occasion t to occasion $t + 1$.

Fig. S2. Seasonal variation in emigration rates.

Fig. S3. Seasonal variation of capture rates in infected birds.

Fig. S4. Seasonal variation of capture rates in non-infected birds.

Table S1. Data summary.

Table S2. Goodness of fit tests.

Table S3. Model selection step 1.

Table S4. Detailed model selection involved in step 2 and step 3.

Appendix S1. Multistate M-arrays for each age category.