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Dissecting a wildlife disease hotspot: the impact of multiple host species, environmental transmission and seasonality in migration, breeding and mortality

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Avian influenza viruses (AIVs) have been implicated in all human influenza pandemics in recent history. Despite this, surprisingly little is known about the mechanisms underlying the maintenance and spread of these viruses in their natural bird reservoirs. Surveillance has identified an AIV ‘hotspot’ in shorebirds at Delaware Bay, in which prevalence is estimated to exceed other monitored sites by an order of magnitude. To better understand the factors that create an AIV hotspot, we developed and parametrized a mechanistic transmission model to study the simultaneous epizootiological impacts of multi-species transmission, seasonal breeding, host migration and mixed transmission routes. We scrutinized our model to examine the potential for an AIV hotspot to serve as a ‘gateway’ for the spread of novel viruses into North America. Our findings identify the conditions under which a novel influenza virus, if introduced into the system, could successfully invade and proliferate.

1. Introduction

Avian influenza viruses (AIVs) have played a key role in human pandemics over the past century, with avian-derived gene segments identified in all pandemic influenza strains [1–4]. Although primarily an infection of birds, ‘host shifts’ of the virus from birds to humans have been documented [5], causing severe disease or death [6] in some cases. Clearly, understanding the determinants of AIV transmission in their natural reservoir—wild birds—is both important and timely [7], though several factors combine to make this challenging [8,9]:

- (i) *Multiple host species.* AIVs have been isolated from more than 105 bird species from 26 families [9], though most competent hosts are thought to belong to the orders Anseriformes (ducks, geese and swans) and Charadriiformes (gulls, shorebirds and terns). One of the chief complicating aspects of (low pathogenicity) AIV infection in wild birds appears to be the absence of overt clinical symptoms [1], resulting in the need for extensive field sampling of individual birds in order to paint an accurate epizootiological picture in any given population [10].
- (ii) *Seasonal host migration.* The role of multiple hosts in the system also introduces a complex spatial element owing to the idiosyncratic migratory

behaviours of different species. Many bird species are, to some degree, migrants, spending a portion of each year in locations that can be thousands of miles apart. Behaviour at different locations can also vary; mallards (*Anas platyrhynchos*), for example, are observed to be very territorial at their breeding grounds but social at other locations [11]. Despite the potential difficulties this spatial structure generates, migration routes for many species are well documented and can provide information on the timing and location of interspecific mixing [12]. The role of migration in disease spread has come into focus lately [13], with recent work suggesting that birds with asymptomatic AIV infections could be responsible for the spread of H5N1 across countries or even continents [14]. Observations in the field—such as that of migrating wild geese in China and Tibet wintering close to their domestic counterparts [15]—support this hypothesis.

- (iii) *Virus diversity*. AIVs demonstrate extensive genetic variation. They are classified according to two surface glycoproteins—haemagglutinin ('H') and neuraminidase ('N')—with 144 possible subtypes in total (combinations of H1–H16 and N1–N9) [16]. The duration and extent of protective immunity following infection are open questions, with experimental work confined to short-term studies [17,18].
- (iv) *Mixed transmission mechanisms*. Finally, it is increasingly thought that AIVs boast two distinct transmission routes in waterbirds. In addition to the essentially direct faecal–oral mechanism (short time scale; susceptible and infected birds in close proximity) [1], an environmental component to transmission has been identified [19–24]. Influenza A viruses have been shown to persist in water for several months [20,21], leading to indirect transmission chains via the environment that occur over a much longer time scale than faecal–oral transmission. On this time scale, transmission could occur between species that never directly interact but instead share a location each occupies at a different time during the year [10].

These complexities converge in Delaware Bay, USA, and, together with concerted surveillance efforts at this site, offer a unique opportunity to study the epizootiology of AIVs in their natural hosts.

Delaware Bay is a site of hemispheric importance for shorebirds [25], with bird densities reaching as high as 210 birds per square metre [26]. Multiple species migrate to Delaware Bay throughout the year [27], making it a pivotal site for understanding bird ecology. In particular, Delaware Bay has previously been identified as a 'hotspot' for AIVs in shorebirds, with estimated average prevalence from 1998 to 2008 about 50 times greater than for all other surveillance sites worldwide [26]. This observation needs to be explained because it suggests that Delaware Bay may act as a place where novel avian viruses can amplify and subsequently spread in North America.

A factor that many consider key to the high AIV prevalence in shorebirds (in particular, in ruddy turnstones (*Arenaria interpres*)) in Delaware Bay is the abundance of horseshoe crabs (*Limulus polyphemus*) there [26]. Every year, thousands of shorebirds congregate on the beaches of Delaware Bay and feast on the horseshoe crab eggs, laid in their millions each spring [28]. The shorebirds in Delaware Bay depend almost entirely on horseshoe crab eggs to

refuel them during their spring migration [26]. This complete dependence makes them vulnerable to horseshoe crab population sizes, which have been declining in recent years [29]. This dependence is an important consideration, because of the role it will play both in shorebird population sizes (declines have already been noticed in Delaware Bay [29]) and on the AIV prevalence levels in these species.

The initiation of the AIV prevalence peak observed in Delaware Bay in ruddy turnstones is not known. As studies suggest that AIV is not present year round in this species [26], it may therefore rely on the maintenance cycle of AIVs driven in part by resident and migratory ducks. To understand this system and examine its consequences for invasion of novel viruses, we develop a multi-host, multi-site AIV transmission model, with parameters estimated using existing prevalence data, that represents a simplified version of the interactions in Delaware Bay. We focus on three host species that we consider key to understanding transmission dynamics in Delaware Bay, with each interacting with the Delaware Bay environment for different periods of time during the year. All three species return a high average percentage of positive AIV isolations, either globally [9] or within Delaware Bay [26]. The three hosts, and their interaction with Delaware Bay, are: (i) *ruddy turnstones* (a short-term visitor to Delaware Bay)—of the shorebirds tested for AIV in Delaware Bay, this species most frequently returns positive results [26]; (ii) *American black ducks* (*Anas rubripes*; 'resident ducks' in our system)—a locally breeding species with resident and migratory birds present throughout the year; (iii) *mallards* ('migrating ducks' in our system), a long-term visitor to Delaware Bay—and a species with one of the highest reported percentages of AIV isolations [9]. The migration biology of this system, and the wintering/breeding sites included in the model for one or more of the migrating species, is illustrated in figure 1.

In addition to multiple host species, we consider mixed transmission dynamics and species-specific seasonality in breeding, hatching, mortality and migration. Our results show that the source and route of AIV infection varied throughout the year, depending on season-specific migration to and from Delaware Bay and which species were reproducing. Motivated by recent declines in horseshoe crab abundance [29], the model is studied to examine the consequences of continuing declines in resources (horseshoe crabs) for the ruddy turnstone population and the broader impact this has on AIV transmission in Delaware Bay. To quantify the chance that any future introduction of a novel strain to Delaware Bay will invade, and to determine the window of opportunity during which invasion is most probable, we calculated the local Lyapunov exponent (LLE; see S9 in the electronic supplementary material for a description). These results show that invasion is most likely when ruddy turnstones are in Delaware Bay or when hatching is occurring in any species.

2. The model

We address AIV transmission dynamics in Delaware Bay by constructing a deterministic, continuous time, three-host, susceptible–infectious–recovered–susceptible (*SIRS*) model. The key model ingredients are outlined below.



Figure 1. Migratory ecology of the simplified three-host Delaware Bay system. The migration routes for ruddy turnstones and mallards are shown, with the inset showing the timing of their presence in Delaware Bay. Also marked in the inset (in dark blue) is the breeding season of resident ducks.

2.1. Seasonal migration

Two of the host species—ruddy turnstones and mallards—follow specific migration patterns. Ruddy turnstones are on their wintering grounds from September to May, in Delaware Bay for the majority of May and on their breeding grounds the rest of the year. Mallards winter in Delaware Bay from October to February and spend the rest of the year on their breeding grounds. The third host species—American black ducks—remains in Delaware Bay throughout the year. Details of the migration parameters are presented in the electronic supplementary material, §S3.

2.2. Seasonal hatching

The pulsed influx of susceptible juveniles is known to be important for transmission dynamics, both in the context of AIVs in bird populations [1] and more generally [30,31]. Therefore, we consider season-specific hatching rates in our model. Duck hatching rates are constant for a quarter of the year (during the hatching season) and zero otherwise [11,32]. The hatching season for ruddy turnstones is shorter, lasting

for a tenth of a year [33]. These parameters are presented in the electronic supplementary material, §S4.

2.3. Seasonal mortality

In duck species, hunting is thought to be a significant contribution to annual mortality [34]. We include this element of duck life history by increasing the mortality rate in both duck species during the hunting season (October–January) [35] (see §S4 in the electronic supplementary material for parameter details).

2.4. Ruddy turnstone feeding ecology

While in Delaware Bay, ruddy turnstones feed on horseshoe crab eggs buried in high concentrations on coastal, sandy beaches [36]. Eggs are usually buried 15–20 cm beneath the surface, but are displaced by both other spawning crabs and tide movements [29,37]. Without a sufficient supply of horseshoe crab eggs, shorebirds are less likely to successfully complete their migration and breed [29]. To model this, we made the ruddy turnstone hatching rate

dependent on the number of horseshoe crabs, E , as shown in the electronic supplementary material, equation (S1a). During our numerical analysis, we varied the number of horseshoe crabs, to assess how resource limitation affects AIV prevalence. We began with a large value of E and ran the numerical model for 500 years, retaining the peak prevalence values from the last 50 years. Using the final class sizes as our new initial conditions, we reset E to a smaller value and ran the numerical model. We repeated this for 100 values of E .

2.5. Direct transmission

Within each species, the direct transmission rate varies through the year. The contact rate in duck species is assumed to be lower immediately before and at the start of the hatching season, when birds form mating pairs and become aggressive towards conspecifics (thereby interacting less than at other times during the year) [11,32]. Transmission among ruddy turnstones is assumed to be low all year except for when they are in Delaware Bay, where contact rates are greatly increased (based on density estimates [26]). We use square wave functions to represent these variations. Between-species transmission rates are set to either zero or a non-zero constant, depending on the time of year. The transmission matrix and parameters are given in the electronic supplementary material, §S2. As supported by empirical evidence [38] and previous theoretical studies, we assume density-dependent transmission [39,40].

2.6. Environmental transmission

We include classes in our model for the environmental reservoir at each location, as in Breban *et al.* [39]. Virus is assumed to be shed at a constant rate into the environment by infected birds and to decay at a time-dependent rate, owing to temperature variation at the different locales (see the electronic supplementary material, §S5, for details). The virus concentration in the environment is represented by V in the model. The environmental transmission term represents the rate at which a susceptible bird consumes virions (ρSV), modified by a probability of infection term, $\rho V / (\rho V + \kappa)$. Hence, ρV is the amount of virus consumed per unit time, κ represents the ID_{50} (virus dose that has a 50% chance of generating an infection) and this expression determines infection probability per unit time. We estimate the value of ρ by fitting the model to existing prevalence data, as shown in the electronic supplementary material, §S7. Virus decay parameters are given in the electronic supplementary material, §S5.

2.7. Immunity

Our transmission model permits loss of immunity. Best-fit parameter estimates (see the electronic supplementary material, §S7) yielded a mean duration of immunity of approximately six months, consistent with experimental data suggesting that antibodies decline to undetectable levels within about eight months [18]. We assume the average duration of immunity in ruddy turnstones to be 1 year, based on empirical evidence that shows the majority of birds annually arrive in Delaware Bay seronegative and convert while there [41].

The system of equations describing a single-host model is given in (2.1). The full model and the seasonal parameters are given in the electronic supplementary material,

§S1–S5; parameter estimates for all hosts are given in table 1.

$$\frac{dS}{dt} = b(t)N - \beta(t)SI - \rho \left(\frac{\rho V}{\rho V + \kappa} \right) SV + \epsilon R - \mu(t)S, \quad (2.1a)$$

$$\frac{dI}{dt} = \beta(t)SI + \rho \left(\frac{\rho V}{\rho V + \kappa} \right) SV - (\gamma + \mu(t))I, \quad (2.1b)$$

$$\frac{dR}{dt} = \gamma I - (\epsilon + \mu(t))R \quad (2.1c)$$

$$\text{and} \quad \frac{dV}{dt} = \omega I - \eta(t)V. \quad (2.1d)$$

Here, N represents the total population size and is given by $N = S + I + R$ (I is the infected class and R the immune class). We derive an expression for the effective basic reproductive value, R_0^e [47,48], assuming no seasonality (all parameters are constant) and the approximation $\rho V / (\rho V + \kappa) = A$ (A const.). For comparison, we also present R_0^e when this assumption is not made. We can extend this to include the seasonally varying terms in our model by defining $R_0^e(t)$ as the R_0^e value at time t when a single infected individual enters an otherwise susceptible population [49]. The expression for $R_0^e(t)$ from equations (2.1) is

$$R_0^e(t) = \begin{cases} \left(\beta(t) + \frac{\omega \rho A(t)}{\eta(t)} \right) \frac{S(t)}{(\gamma + \mu(t))} & \text{with environmental transmission,} \\ \frac{\beta(t)S(t)}{(\gamma + \mu(t))} & \text{without environmental transmission.} \end{cases} \quad (2.2)$$

The $R_0^e(t)$ values that apply to each species are given in the electronic supplementary material, §S6. In §4, we use this time-varying $R_0^e(t)$ to quantify the relative effect of interspecies mixing on AIV transmission dynamics in Delaware Bay.

3. The epizootiological data

The ideal data for fitting the model would be of high temporal resolution, with large numbers of samples at each time point, and would exist for multiple species across their migration ranges. Unfortunately, these data do not as yet exist; so we take a pragmatic approach and available data to guide model parametrization.

Two sources of surveillance data were used for model fitting. The first comprises published prevalence estimates from Stallknecht & Shane [50]. These data come from a variety of sources and studies, incorporating different bird-trapping methodologies and virus isolation techniques, but together represent the best source of information regarding prevalence cycles in dabbling ducks in North America. We apply least-squares estimation to these data to quantify four parameters for migrating ducks, and assume that the same values hold for resident ducks (the methodological detail is presented in the electronic supplementary material, §S7). Similarly, in the absence of independent information on consumption rate or infection shape parameter in ruddy turnstones, we take these values to be the same as those used for the duck species.

The second set of data are published here for the first time and come from the US Early Detection System for Highly Pathogenic Avian Influenza in Wild Birds (data collection described in Deliberto *et al.* [51]). These data were collected in Delaware

Table 1. Standard parameter values for each host species. The superscripts m,r,u stand for migrating ducks, resident ducks and ruddy turnstones, respectively.

parameter	symbol	value/range	unit	source
<i>mallards</i>				
direct transmission (baseline)	β_0^m	0.01	year ⁻¹	parametrization
amplitude of seasonality	β_1^m	0.75		parametrization
birth rate	b_0^m	2	year ⁻¹	[42]
average death rate	$\bar{\mu}^m$	0.5	year ⁻¹	[42]
recovery rate	γ^m	52	year ⁻¹	[1]
loss of immunity	ϵ^m	2.004	year ⁻¹	parametrization
consumption rate	ρ^m	1.3804×10^{-12}	year ⁻¹	parametrization
infection shape parameter	κ	100	EID ₅₀	[43]
shedding rate	ω^m	10^{12}	EID ₅₀ year ⁻¹	[44]
persistence	η	4.9–42.6	year ⁻¹	[19]
<i>American black ducks</i>				
direct transmission (baseline)	β_0^r	0.01	year ⁻¹	assumed = β_0^m
amplitude of seasonality	β_1^r	0.75		assumed = β_1^m
birth rate	b_0^r	2	year ⁻¹	[34]
average death rate	$\bar{\mu}^r$	0.5	year ⁻¹	[34]
recovery rate	γ^r	52	year ⁻¹	[1]
loss of immunity	ϵ^r	2.004	year ⁻¹	assumed = ϵ^m
consumption rate	ρ^r	1.3804×10^{-12}	year ⁻¹	assumed = ρ^m
infection shape parameter	κ	100	EID ₅₀	[43]
shedding rate	ω^r	10^{12}	EID ₅₀ year ⁻¹	[44]
persistence	η	13.9–42.6	year ⁻¹	[19]
<i>ruddy turnstones</i>				
direct transmission (baseline)	β_0^u	0.005	year ⁻¹	assumed = $0.5 \times \beta_0^m$
amplitude of seasonality	β_1^u	0.5		estimated
birth rate	b_0^u	1.5	year ⁻¹	[45]
death rate	μ^u	0.15	year ⁻¹	[45]
recovery rate	γ^u	52	year ⁻¹	[46]
loss of immunity	ϵ^u	1	year ⁻¹	[41]
consumption rate	ρ^u	1.3804×10^{-12}	year ⁻¹	assumed = ρ^m
infection shape parameter	κ	100	EID ₅₀	—
shedding rate	ω^u	10^{10}	EID ₅₀ year ⁻¹	D. Stallknecht, estimate based on unpublished data (2007–2008)
persistence	η	1.6–167.9	year ⁻¹	[19]
predator shape parameter	θ	10^{-4}	year ⁻¹	—
number of horseshoe crabs	E	$1–10^5$		—

during the winter months for three consecutive years (2007–2010) and are presented in the electronic supplementary material.

4. Results

Our model explains that AIV dynamics in Delaware Bay are shaped by a combination of factors. The role of the environmental reservoir is apparent in a comparison of effective $R_0(t)$ values ($R_0^e(t)$) for species interacting in Delaware Bay in both the presence and absence of an environmental component (figure 2a), demonstrating that an environmental

reservoir increases R_0^e . When migrating ducks and resident ducks are initially together in Delaware Bay, $R_0^e(t) > 1$ regardless of the environmental reservoir, although it is much higher when the environmental component is included. During the post-breeding period in resident ducks, inclusion of an environmental component produces $R_0^e(t) > 1$ (without it, $R_0^e(t) < 1$ during this period). Equally apparent is the role of interactions between host species—in particular, the interaction between ruddy turnstones and resident ducks. When considering $R_0^e(t)$ for each species if modelled individually (i.e. as in (2.2)), $R_0^e(t)$ for resident ducks when the ruddy turnstones are present in Delaware Bay is less than 1. However,

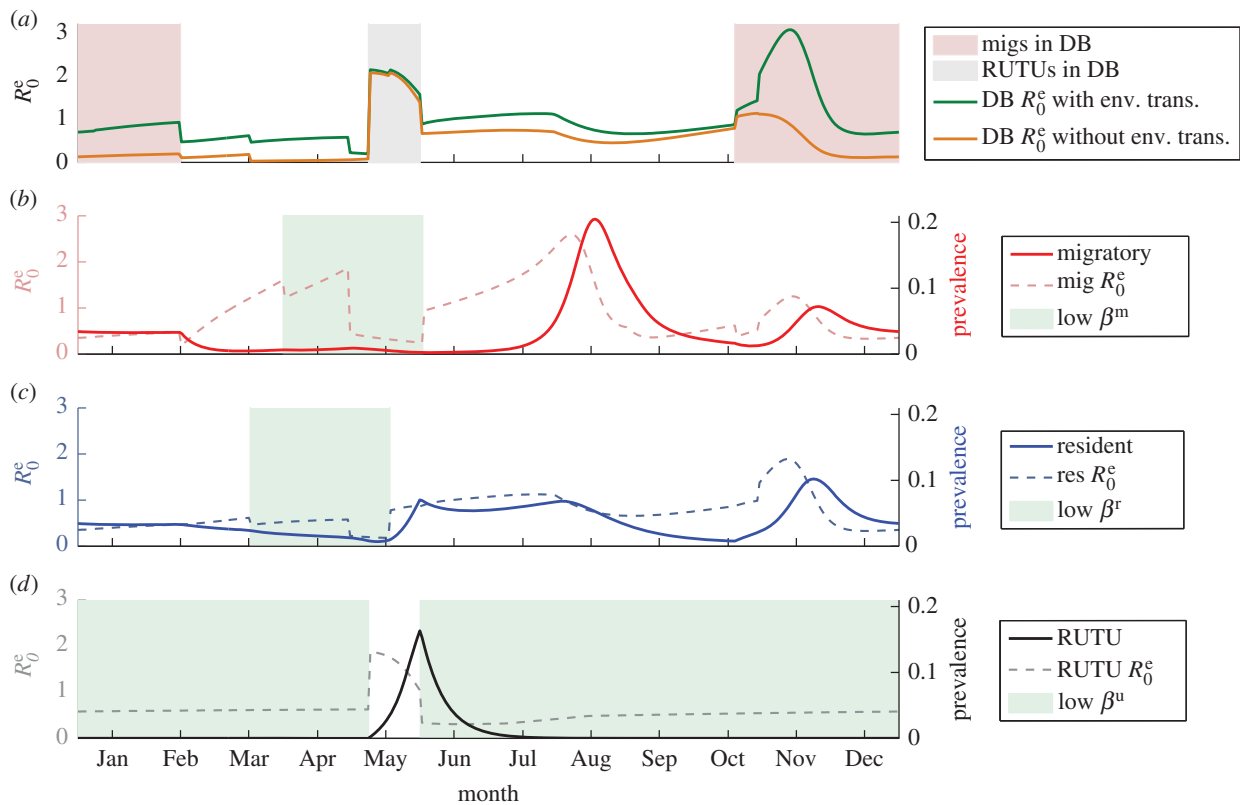


Figure 2. Prevalence curves from the multi-host model against effective $R_0(t)$ values [47]. (a) $R_0^e(t)$ values for species in Delaware Bay both with and without environmental transmission; (b) the migratory duck (mig) prevalence alongside the effective $R_0(t)$ for migratory ducks individually; (c) the resident duck (res) prevalence and the effective $R_0(t)$ for resident ducks individually; and (d) the ruddy turnstone (RUTU) prevalence with the effective $R_0(t)$ for ruddy turnstones individually. In (b)–(d), the times of low transmission for the species shown are shaded and the R_0^e values are shown with environmental transmission. We assume the population size for each host to be 10 000 [33,39,52]. Initial conditions in the duck hosts are $S(0) = 225$, $I(0) = 1$, $R(0) = 9774$ (robustness to initial conditions is shown in the electronic supplementary material); for the ruddy turnstones they are $S(0) = 9999$, $I(0) = 1$, $R(0) = 0$. We assume that some virus is present initially at Delaware Bay and the duck breeding grounds, with $V(0) = 100$. We assume no virus is initially present at the ruddy turnstone wintering and breeding grounds [26,50].

the interaction between resident ducks and ruddy turnstones is such that $R_0^e(t)$ for the two species is greater than 1, and a peak in prevalence in resident ducks is observed. This is seen in figure 2c, which shows both the prevalence curve and the individual $R_0^e(t)$ for resident ducks against time. The impact of the interaction between both duck species is less obvious, as their individual $R_0^e(t) > 1$ during the timing of their interaction (figure 2b,c). However, the combined $R_0^e(t)$ is a lot greater than the individual ones, contributing to the size of the prevalence peak observed.

We can further use the model to determine the dominant transmission route throughout the year in each species. Each panel in figure 3 shows the prevalence for a particular host species, with a background that shows the proportion of cases generated via each transmission route throughout the year. Figure 3a shows the prevalence curve and contribution of each transmission route for migratory ducks. The main peak in prevalence in this host occurs prior to its arrival in Delaware Bay, after the influx of new susceptibles has occurred in the hatching season. Our model predicts that a second, smaller peak in prevalence is initiated by their arrival in Delaware Bay and mixing with resident ducks. The contributions from each of the transmission routes indicate that, outside Delaware Bay, the majority of infections in migrating ducks are caused by either within-species transmission or environmental transmission at different times of year. In Delaware Bay, our model suggests that within-species and between-species interactions contribute almost equally to new cases in both migrating and resident (figure 3b) ducks,

although environmental transmission plays the largest role, accounting for approximately 80 per cent of cases during this time.

Figure 3b shows the equivalent curve for resident ducks. The numerical results suggest that three prevalence peaks occur every year. The model results demonstrate that the first peak results from the interaction between resident ducks and ruddy turnstones, and the final peak is due to the interaction between resident ducks and migrating ducks. The middle peak leads on from the first peak and is a response to the influx of susceptibles during the breeding season. These predictions suggest that the non-zero prevalence early in the year in both duck species is a consequence of the loss of immunity in ducks while migrating ducks are still in Delaware Bay. When the resident ducks are alone in Delaware Bay, within-species interactions account for between 60 and 80 per cent of transmission during the summer months, but environmental transmission is the dominant transmission route early in the year. The influence of ruddy turnstones is seen immediately before the first peak, when almost all transmission occurs via this species. Similarly, the role of migrating ducks is clear as they spark the peak in prevalence in residents, causing approximately 50 per cent of new cases as they arrive. The majority of transmission during this time period, however, is due to the environmental reservoir.

Figure 3c displays the cycle in ruddy turnstones, with peak prevalence occurring at the end of their stay in Delaware Bay. In assessing the contribution of each transmission

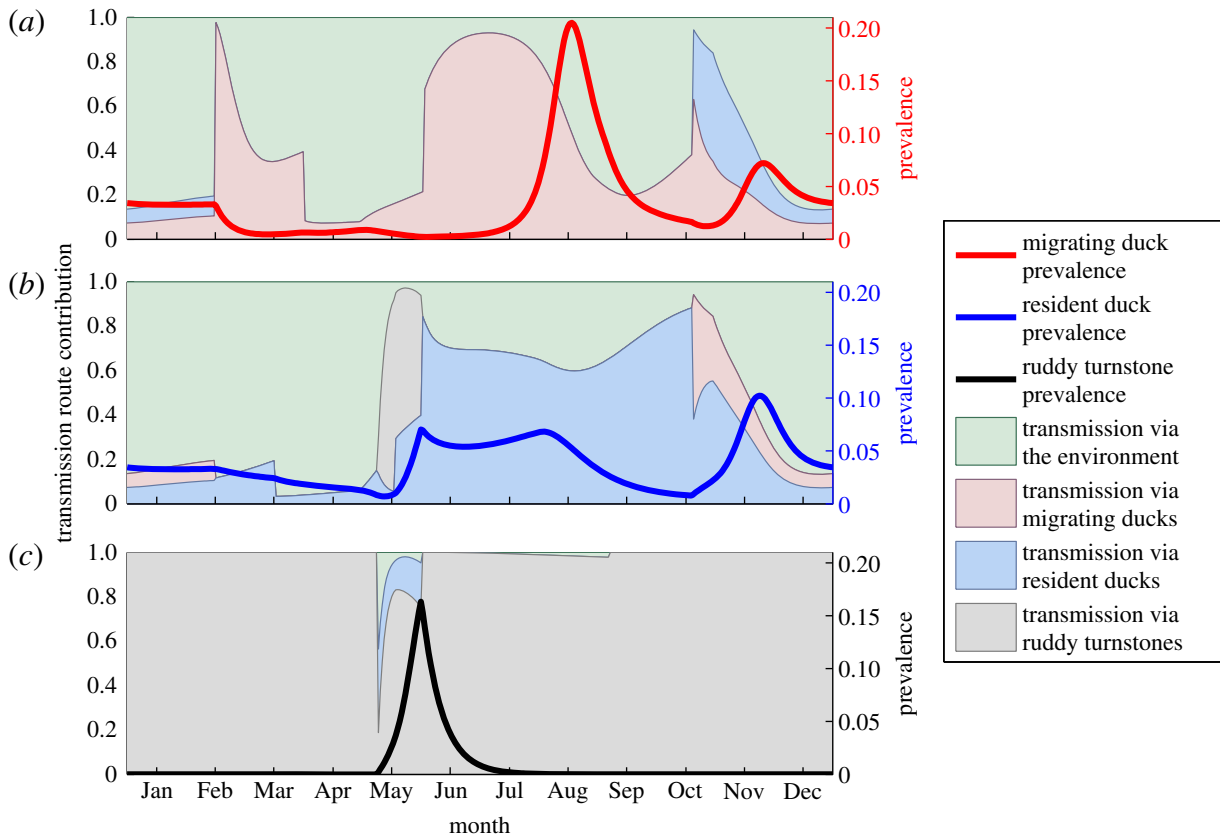


Figure 3. Prevalence and incidence plots for the multi-host model showing the proportion of infections by their origin, alongside the prevalence curve, over time. (a) The migratory ducks, (b) the resident ducks and (c) the ruddy turnstones.

route in this species, it can be seen that they show very little dependence on other species, with almost all transmission through within-species interactions. Notably, spikes in the proportion of infections transmitted via the environment and through interspecies interactions occur immediately before the prevalence peak, implying that these two factors are initiating their prevalence peaks. However, comparison of figure 3*b* and 3*c* indicates that the ruddy turnstones are a much greater influence on AIV prevalence among resident ducks than vice versa.

We can establish two results from the prevalence curves for each species and the contribution of the various transmission routes. First, it is clear that the presence of resident ducks in the model is a key factor in the persistence of AIV transmission in Delaware Bay. Further results presented in the electronic supplementary material (figure S13*b*)) provide evidence of this, with outbreaks of AIV no longer occurring in ruddy turnstones when resident ducks are removed from the model. Moreover, these results (figure 3) show how important environmental transmission is, particularly in the case of the duck species. For much of the year in these species, transmission from the environmental reservoir is the dominant transmission route. We also find that interactions between the resident ducks and ruddy turnstones play a key role in the prevalence curves for each of these species, apparently providing the impetus for a peak in prevalence in both species.

We conducted a thorough sensitivity analysis on several of the model parameters, with the results presented in the electronic supplementary material, §S8. We found that the results are qualitatively very similar to the results presented here. The effect of changing the resident or ruddy turnstone

population sizes is also presented in the electronic supplementary material, §S8, where we show that a small resident population has very little impact on the prevalence peaks in the migrating ducks but does change the height of the prevalence peak in ruddy turnstones. Similarly, altering the size of the ruddy turnstone population changes the peak prevalence in ruddy turnstones. Furthermore, we evaluated the role of each of the individual species in the system as a whole, by removing each in turn and considering the resulting prevalence curves and transmission routes (see the electronic supplementary material for sensitivity analyses).

To systematically assess the contribution of key epizootiological parameters in our model output, we carried out a sensitivity analysis using Latin hypercube sampling (LHS). Specifically, we assigned a broad range of possible values to critical parameters—average direct transmission rate in the duck species; amplitude of seasonality in the aforementioned transmission rates; duration of immunity in the duck species; recovery rate in all species; consumption rate in all species; and number of horseshoe crabs—and generated 100 parameter sets using LHS (further detail is given in the electronic supplementary material, table S1). For each parameter set, peak prevalence in ruddy turnstones was noted to determine how it is influenced by changes in parametrization. We also compared these results with the peak prevalence found when only one of the parameters in question was allowed to vary and all others remained as given in table 1.

The results show (figure 4) that parameters that are more indirectly linked to peak prevalence in ruddy turnstones in Delaware Bay (such as amplitude of seasonality of direct transmission in the two duck species) have a smaller effect over their range than those that have a direct influence on

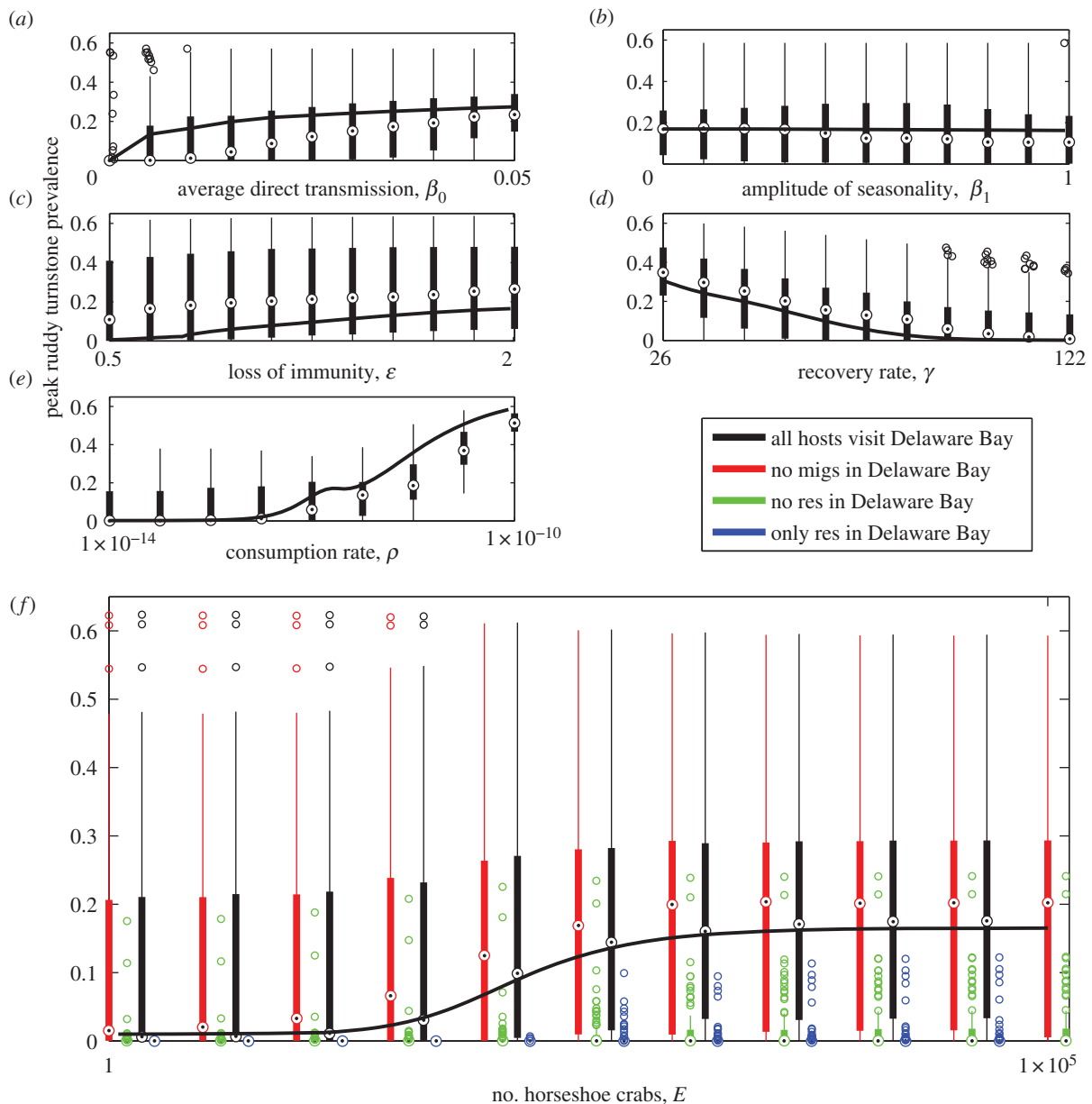


Figure 4. Effects of parameter variation on peak AIV prevalence in ruddy turnstones in Delaware Bay, using Latin hypercube sampling (LHS) across key epidemiological parameters. Each panel shows the results of varying one of these parameters and maintaining all other parameters as given in table 1 (some exceptions are required—see the electronic supplementary material for details) as a black line. The LHS for each parameter is then shown as a boxplot. (a) The results as the direct transmission rate in ducks varies; (b) the results as the amplitude of seasonality in the ducks' direct transmission rate varies; (c) the results from varying the duration of immunity of ducks; (d) the results as the recovery rate varies in all three species; (e) the results if the consumption rate varies in all species; and (f) how peak prevalence varies as the number of horseshoe crabs is varied. The range for each of the parameters used in the LHS is given in the electronic supplementary material, table S1.

AIV epizootiology in ruddy turnstones (e.g. recovery rate, consumption rate). In these two cases, mean peak prevalence in ruddy turnstones changes dramatically over the range of values tested (varying between 0.07 and 0.31 for the recovery rate and between 0.07 and 0.48 for the consumption rate).

Using LHS alongside different combinations of host species passing through Delaware Bay provides an insight into the role of multiple host species in the model system (figure 4f). Host species combinations are found to have a significant effect on peak prevalence in ruddy turnstones in Delaware Bay—specifically, interaction with resident ducks in the system is crucial for non-zero peak prevalence in ruddy turnstones. If they are either removed from the model or ruddy turnstones do not travel through Delaware Bay and so do not interact with them, mean peak prevalence in ruddy turnstones is zero (note that if ruddy turnstones do

not travel through Delaware Bay, peak prevalence at the same time of year is shown instead).

Results shown so far pertain to the known epizootiological situation. For a more prospective use of the model, we now turn to a key component in this system that is exhibiting a long-term trend—the number of horseshoe crabs. We find that the number of horseshoe crabs present can exert great influence on the prevalence curves for both the resident ducks and ruddy turnstones, with peak AIV prevalence in ruddy turnstones decreasing as horseshoe crab numbers, E , decline. The sharpest reduction occurs in the region $30 < E < 1000$. Figure 5 shows this trend, with insets that show changing prevalence curves for all three hosts with ($E = 10$) and without ($E = 10^5$) resource limitation. Furthermore, we note that the decline in AIV prevalence in ruddy turnstones leads to an increase in the tallest prevalence peak in resident

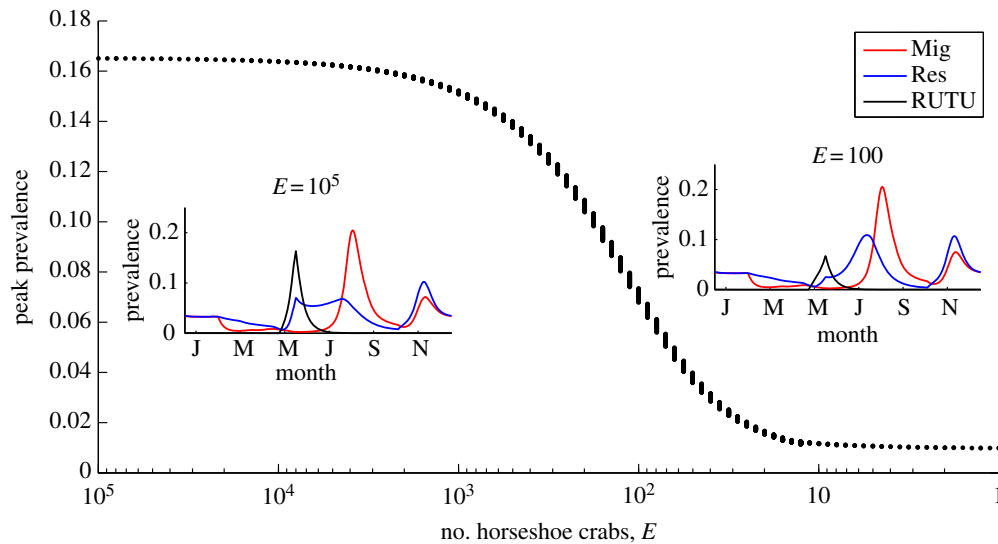


Figure 5. Prevalence curves for reduced resources for the ruddy turnstones. The main figure shows the peak prevalence in ruddy turnstones as the number of horseshoe crabs (E) decreases for a ruddy turnstone population of 10 000, using updated initial conditions in the ruddy turnstone population. The inset figures show prevalence curves predicted by the model for two different values of E . Mig, migratory ducks; Res, resident ducks; RUTU, ruddy turnstones.

ducks, as their prevalence curve becomes more like that of the migrating ducks.

A potentially important dimension to the identification of Delaware Bay as an avian influenza hotspot concerns the likely role played by this site in the successful invasion of any novel AIV strain. That is, are there specific windows of vulnerability during which amplification of an introduced virus is predicted? We answer this question by calculating a dynamic and time-dependent measure of pathogen invasion potential, specifically the LLE (described in the electronic supplementary material, §S9). A negative LLE indicates that any perturbation (resulting from a virus introduction) will decay in the short term, while a positive exponent signals locally exponential growth [53]. From this metric, we find that seasonal hatching and the ruddy turnstones' stay in Delaware Bay are the two key determinants of the sign of the LLE, with the four-month period between May and August, covering these events, as the period during which Delaware Bay can act as a gateway. This is indicated by a positive LLE (figure 6), with especially large amplification potential during the ruddy turnstones' sojourn in Delaware Bay. This result helps establish the contribution of seasonal breeding and seasonal migration (in ruddy turnstones) to the definition of Delaware Bay as a 'hotspot'.

5. Discussion

Delaware Bay has long been recognized as an important and anomalous site in AIV epizootiology, although the reasons behind this discrepancy have not been fully understood. We have dissected this vital question, pinpointing some of the key mechanisms that are likely to contribute to the AIV dynamics observed at the site.

We parametrized our model using available data (as described in §3). Unfortunately, the prevalence curve for mallards does not come from a single data source but is the amalgamation of a variety of studies conducted in different months. These independent studies were carried out over different time periods and used different virus detection and isolation techniques, but together represent what is

known about AIV prevalence in migrating mallards. We used these data together with prevalence estimates provided by the US Department of Agriculture (see §3 and the electronic supplementary material for greater detail), and the resulting model trajectory is therefore a compromise between these independent data sources. We minimized the sum of squared errors (see the electronic supplementary material for methodological details) to determine the best fit parameters—owing to the fragmented nature of the mallard data, it was not possible to adopt more elaborate statistical inference methods. We found that our parametrized model successfully captures key seasonal trends of the data, but systematically under-represents prevalence—particularly in the resident ducks. There are a number of possible reasons for this. Firstly, the fact that our parameter estimates are a compromise from fitting the model to two different data sources is likely to play a role. Secondly, the prevalence levels observed in PCR-based isolation data (for American black ducks in Delaware) are surprisingly high—understanding why presents an interesting topic for further work. Finally, it is possible that our model may not be capturing an element of the system that is driving the high prevalence levels observed. Uncovering whether this is the case, and what this element could be, is likely to be driven by long-term surveillance data from these birds—data that do not currently exist. However, given the ability of our model to successfully capture the seasonal trends present in both datasets, we are still able to draw useful inferences from our results.

The model analysis indicates that prevalence peaks occurring in Delaware Bay, in any of the species represented in our model, are a result of several integrated factors. The migrating ducks have an annual, pre-arrival peak in prevalence owing to both direct within-species transmission and transmission from the environmental reservoir. This peak in prevalence is succeeded by another through interactions with the resident ducks in Delaware Bay. Equally, the ruddy turnstones are capable of driving their own prevalence peaks, but these are initiated by both the environmental reservoir in Delaware Bay and interaction between ruddy turnstones and resident ducks. The model shows that

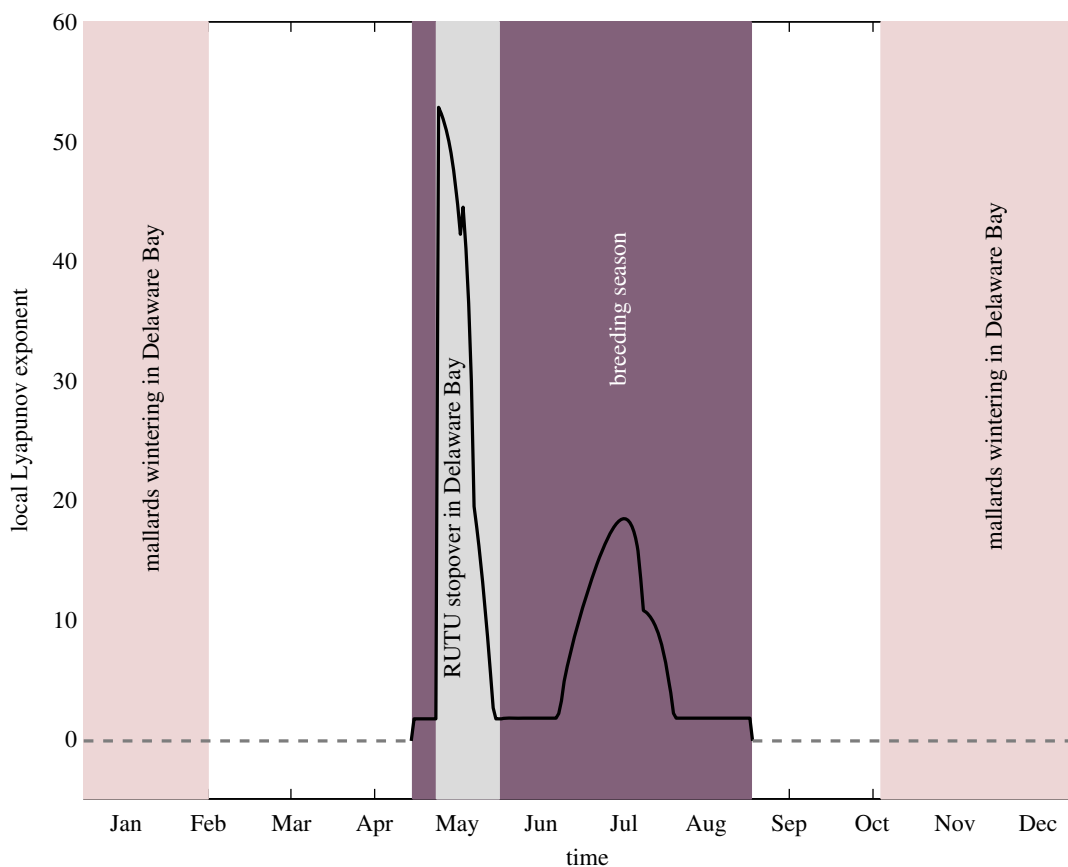


Figure 6. The local Lyapunov exponent (solid line, positive; dashed line, negative) against time over a year. The shaded areas show when the migrating ducks are in Delaware Bay, when the ruddy turnstones are in Delaware Bay and when hatching is occurring in the system (of any host). The x -axis ticks mark the midpoint of each month.

prevalence peaks in resident ducks are sparked by the arrival of either the ruddy turnstones or the migrating ducks, and, in the summer months, maintained by the within-species transmission in resident ducks. In particular, this analysis suggests that both between-species interactions and transmission via the environment are important elements in the determination of Delaware Bay as a hotspot, as they are so influential in transmission. In particular, the importance of between-species interaction is highlighted when comparing peak prevalence in ruddy turnstones as different combinations of host species are included in the model. We find that, without the key interaction between ruddy turnstones and resident ducks, peak prevalence remains zero even when other epidemiological parameters are allowed to vary (figure 4*f*).

Our model predicts a multi-peaked prevalence curve in ducks, with the initiation of each peak through the year attributable to a different source. In our model, resident ducks display a peak in prevalence as migrating ducks arrive in Delaware Bay, followed by non-zero prevalence immediately prior to the departure of the migrating ducks. Notably, similar prevalence levels have been observed at the same time of year in data collected from Europe [54]. The summer peaks that occur in resident ducks cannot yet be verified as the necessary data are currently lacking, but our model suggests that they are a result of either interactions with the ruddy turnstones or the influx of new susceptibles in the post-breeding period.

The effective $R_0(t)$ values from the model offer an explanation for Delaware Bay as an AIV hotspot. A peak in AIV prevalence could occur in Delaware Bay while either of the migrating species is present or briefly during the summer months as a result of the influx of susceptible resident

ducks. Outside Delaware Bay, the effective $R_0(t)$ for ruddy turnstones is too low for prevalence peaks to occur, but migrating ducks maintain a sufficiently large effective $R_0(t)$ to admit prevalence peaks, with annual peaks in prevalence prior to their arrival in Delaware Bay. The arrival of either of the other species in Delaware Bay increases the effective $R_0(t)$ value there and prompts a peak in prevalence in the resident ducks. The effective $R_0(t)$ values show the impact of heterospecific interactions, which greatly increase the effective $R_0(t)$ value.

The model predicts that peak prevalence in ruddy turnstones decreases as horseshoe crabs decrease in abundance. An annual prevalence cycle is apparent until the number of horseshoe crabs is so limited that ruddy turnstone populations can no longer be supported in Delaware Bay. The impact of this on the system is not straightforward. Although prevalence in ruddy turnstones declines, it leads to peak annual prevalence in the resident duck population increasing over time. This is a result of the resident duck prevalence curve losing its May–June peak and instead developing a prevalence curve similar to that in migrating ducks, with one main (post-hatching) peak in the year. Surveillance will need to be ongoing and long term to identify this consequence of decreasing ruddy turnstone prevalence.

We explored the question ‘when could a novel avian virus invade North America?’, by determining the time-dependent invasion potential in our system, as characterized by LLEs. Our model analysis established when Delaware Bay may serve as a potential amplification site for a new AIV subtype. Specifically, our results show that the two biggest predictors of this are when the ruddy turnstones are in Delaware Bay or

during the hatching seasons, when there is an influx of susceptible. Were it to occur while ruddy turnstones are in Delaware Bay, immediate transmission to the resident duck population would be likely. A successful invasion during the hatching season may have less wide-reaching consequences depending on the physiological effects of infection on its host, in particular whether migratory traits and, therefore, the spread to other host species and locations are affected. Identifying the potential origin of such a virus is beyond the scope of this work, but would contribute vital information to the role of Delaware Bay in the spread of AIVs. This result offers two more components of the system that promote Delaware Bay's status as a hotspot—seasonal migration and seasonal breeding.

Our work has provided insight into potentially important ecological parameters affecting AIV ecology in Delaware Bay. The combination of model analyses attests to the synergistic contributions of multiple host species, migration biology, virus kinetics in the environment and seasonal shifts in direct transmission in generating an AIV transmission hotspot. Along with Delaware Bay, four other sites in North

America are key shorebird sites (Copper River Delta, Alaska; Gray's Harbor, Washington; Bay of Fundy, Canada; Cheyenne Bottoms, Kansas [55]) that may also prove to be AIV transmission hotspots. The work presented here provides key factors that contribute to the definition of Delaware Bay as a hotspot, providing vital information that may aid efforts to detect large-scale outbreaks of novel influenza virus in wild-bird populations in the USA.

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S Supplementary Information

S.1 Model equations

We present equations for the full system (See main text for full details). The V , B and A classes are the environmental reservoirs of the virus for Delaware Bay, the migrating ducks' breeding ground, and the ruddy turnstones' wintering and summer grounds combined respectively. The superscripts u, r, m stand for ruddy turnstones, resident ducks and migrating ducks respectively. The superscripts b, db and a refer to different locations in our model, denoting duck breeding grounds, Delaware Bay, and the ruddy turnstones' breeding and wintering grounds respectively.

$$\text{Ruddy Turnstones} \left\{ \begin{array}{l} \frac{dS^u}{dt} = b^u(t) \left(1 - \frac{\theta N^u}{E}\right) N^u + \epsilon^u R^u - \mu^u(t) S^u - (\beta^{uu} I^u + \beta^{ru} I^r) S^u \\ \quad - \rho^{ua} \left(\frac{\rho^{ua} A}{\rho^{ua} A + \kappa}\right) S^u A - \rho^{udb} \left(\frac{\rho^{udb} V}{\rho^{udb} V + \kappa}\right) S^u V, \quad (\text{S1a}) \\ \frac{dI^u}{dt} = (\beta^{uu} I^u + \beta^{ru} I^r) S^u + \rho^{ua} \left(\frac{\rho^{ua} A}{\rho^{ua} A + \kappa}\right) S^u A + \rho^{udb} \left(\frac{\rho^{udb} V}{\rho^{udb} V + \kappa}\right) S^u V \\ \quad - (\gamma^u + \mu^u(t)) I^u, \quad (\text{S1b}) \\ \frac{dR^u}{dt} = \gamma^u I^u - (\epsilon^u + \mu^u(t)) R^u, \quad (\text{S1c}) \end{array} \right.$$

$$\text{Resident ducks} \left\{ \begin{array}{l} \frac{dS^r}{dt} = b^r(t) N^r - (\beta^{rr} I^r + \beta^{ur} I^u + \beta^{mr} I^m) S^r \\ \quad - \rho^r \left(\frac{\rho^r V}{\rho^r V + \kappa}\right) S^r V + \epsilon^r R^r - \mu^r(t) S^r, \quad (\text{S1d}) \\ \frac{dI^r}{dt} = (\beta^{rr} I^r + \beta^{ur} I^u + \beta^{mr} I^m) S^r + \rho^r \left(\frac{\rho^r V}{\rho^r V + \kappa}\right) S^r V - (\gamma^r + \mu^r(t)) I^r, \quad (\text{S1e}) \\ \frac{dR^r}{dt} = \gamma^r I^r - (\epsilon^r + \mu^r(t)) R^r, \quad (\text{S1f}) \end{array} \right.$$

$$\text{Migratory ducks} \left\{ \begin{array}{l} \frac{dS^m}{dt} = b^m(t) N^m + \epsilon^m R^m - \mu^m(t) S^m - (\beta^{mm} I^m + \beta^{rm} I^r) S^m \\ \quad - \rho^{mb} \left(\frac{\rho^{mb} B}{\rho^{mb} B + \kappa}\right) S^m B - \rho^{mdb} \left(\frac{\rho^{mdb} V}{\rho^{mdb} V + \kappa}\right) S^m V, \quad (\text{S1g}) \\ \frac{dI^m}{dt} = (\beta^{mm} I^m + \beta^{rm} I^r) S^m + \rho^{mb} \left(\frac{\rho^{mb} B}{\rho^{mb} B + \kappa}\right) S^m B + \rho^{mdb} \left(\frac{\rho^{mdb} V}{\rho^{mdb} V + \kappa}\right) S^m V \\ \quad - (\gamma^m + \mu^m(t)) I^m, \quad (\text{S1h}) \\ \frac{dR^m}{dt} = \gamma^m I^m - (\epsilon^m + \mu^m(t)) R^m, \quad (\text{S1i}) \end{array} \right.$$

$$\text{Environmental reservoirs} \left\{ \begin{array}{l} \frac{dV}{dt} = \omega^{udb} I^u + \omega^r I^r + \omega^{mdb} I^m - \eta^{db}(t) V, \quad (\text{S1j}) \\ \frac{dB}{dt} = \omega^{mb} I^m - \eta^b(t) B, \quad (\text{S1k}) \\ \frac{dA}{dt} = \omega^{ua} I^u - \eta^a(t) A. \quad (\text{S1l}) \end{array} \right.$$

Here N^i is the total population size, where $N^i = S^i + I^i + R^i$, $i = u, r, m$. The parameters are described in

10 Table 1 and below.

11 S.2 Transmission parameters

12 Transmission within species is denoted by β^{ii} , $i = u, r, m$ and between species as β^{ij} for $i \neq j$, $i, j = u, r, m$.
 13 We assume that the transmission between species (β^{mr} , β^{rm} , β^{ur} and β^{ru}) is the mean of the average direct
 14 transmission rates (e.g. $\beta^{mr} = \beta^{rm} = \frac{1}{2}(\beta_0^m + \beta_0^r)$) within both species. Between-species transmission rates are
 15 non-zero only when the migrating species are present in Delaware Bay and interacting with the resident ducks.
 16 These periods of time are given by the migration parameters in Section S.3. The resulting transmission matrix is

$$\beta = \begin{pmatrix} \beta^{uu} & 0 & \beta^{ur} \\ 0 & \beta^{mm} & \beta^{mr} \\ \beta^{ru} & \beta^{rm} & \beta^{rr} \end{pmatrix} = \begin{pmatrix} \beta^{uu} & 0 & \frac{1}{2}(\beta_0^u + \beta_0^r) \\ 0 & \beta^{mm} & \frac{1}{2}(\beta_0^m + \beta_0^r) \\ \frac{1}{2}(\beta_0^u + \beta_0^r) & \frac{1}{2}(\beta_0^m + \beta_0^r) & \beta^{rr} \end{pmatrix}. \quad (S2)$$

17 We assume perfect mixing between resident ducks and either of the species they interact with; for migratory
 18 ducks this is clear from the similarity of their habitats and behaviours. Between resident ducks and ruddy
 19 turnstones, we base this on research that has shown a variety of habitats are important to shorebird species [1],
 20 offering them the opportunity to interact with other species.

21 The transmission parameters for within-species transmission are given by

$$\beta^{ii} = \beta_0^i \frac{(1 + \beta_1^i \text{Breeding}_i(t))}{\frac{1}{365}((1 + \beta_1^i)D^+ + (1 - \beta_1^i)D^-)}, \quad (S3)$$

22 with D^+ denoting the number of days of high transmission and D^- denoting the number of days of low trans-
 23 mission, $i = u, r, m$. The denominator of (S3) ensures that the average of β^{ii} , β^{ii} , is equal to β_0^i . The ‘Breeding
 24 season’ parameters for the migrating and resident ducks are presented below.

$$\text{Breeding}_m(t) = \begin{cases} -1 & \text{if } 0.25 < t < 0.42, \\ 1 & \text{otherwise.} \end{cases} \quad (S4)$$

$$\text{Breeding}_r(t) = \begin{cases} -1 & \text{if } 0.21 < t < 0.38, \\ 1 & \text{otherwise.} \end{cases} \quad (S5)$$

25 We use the ‘*Mixing_u*’ function (given in Section S.3) in place of a ‘*Breeding_u*’ function in the ruddy
 26 turnstone’s transmission term - this is the period of time ruddy turnstones are present in Delaware Bay and is
 27 given in (S7).

28 S.3 Migration parameters

29 The parameters determining the location of each species at each point in time (i.e. relating to migration patterns)
 30 are given below; the ‘*Mixing*’ and ‘*Mixing_u*’ parameters give the times the migrating ducks and ruddy turnstones
 31 respectively are in Delaware Bay. The ‘*Mixing_u*’ parameter is also used to calculate the within-species direct
 32 transmission rate for ruddy turnstones (as described in Section S.2). The ‘*Summer*’ and ‘*Wintering*’ parameters
 33 give the periods when the ruddy turnstones are on their summer and winter grounds.

$$\text{Mixing}(t) = \begin{cases} 0 & \text{if } 0.125 < t < 0.8, \\ 1 & \text{otherwise.} \end{cases} \quad (S6)$$

$$\text{Mixing}_u(t) = \begin{cases} 1 & \text{if } 0.354 < t < 0.417, \\ 0 & \text{otherwise.} \end{cases} \quad (S7)$$

$$\text{Wintering}(t) = \begin{cases} 0 & \text{if } 0.354 < t < 0.683, \\ 1 & \text{otherwise.} \end{cases} \quad (S8)$$

$$Summer(t) = \begin{cases} 1 & \text{if } 0.417 < t < 0.683, \\ 0 & \text{otherwise.} \end{cases} \quad (S9)$$

34 The shedding and consumption rates for ruddy turnstones and migrating ducks given in (S1) for each location
 35 they visit are also defined using the above parameters (as the above parameters determine which location each
 36 species in for every point in time), so for example shedding by the migrating ducks in Delaware Bay, ω^{mdb} , uses
 37 the $Mixing(t)$ parameter - $\omega^{mdb} = \omega^m Mixing(t)$ (see Table 1 for ω^m ; other shedding and consumption rate
 38 parameters are constructed similarly).

39 S.4 Hatching and mortality parameters

40 The hatching parameter for each species is given below. The superscripts u, r, m denote ruddy turnstones, resident
 41 ducks and migrating ducks respectively. These parameters (in the case of hatching) define when hatching occurs in
 42 the model - i.e. the periods of time when it is non-zero. In the case of the mortality parameters, the ' $Death^i(t)$ '
 43 ($i = m, r$) give the step functions used for the mortality rates in both duck species (so $\mu^m = Death^m(t)$;
 44 $\mu^r = Death^r(t)$).

$$b^m(t) = \begin{cases} b_0^m & \text{if } 0.42 < t < 0.67, \\ 0 & \text{otherwise.} \end{cases} \quad (S10)$$

$$b^r(t) = \begin{cases} b_0^r & \text{if } 0.33 < t < 0.58, \\ 0 & \text{otherwise.} \end{cases} \quad (S11)$$

$$b^u(t) = \begin{cases} b_0^u & \text{if } 0.522 < t < 0.622, \\ 0 & \text{otherwise.} \end{cases} \quad (S12)$$

45 The seasonal mortality rates for the duck species (as a result of hunting [2, 3, 4]) are given below. Subscripts
 46 r, m denote resident and migratory ducks respectively.

$$Death^m(t) = \begin{cases} 0.25 & \text{if } 0.02 < t < 0.83, \\ 1.57 & \text{otherwise.} \end{cases} \quad (S13)$$

$$Death^r(t) = \begin{cases} 0.3 & \text{if } 0.02 < t < 0.83, \\ 1.35 & \text{otherwise.} \end{cases} \quad (S14)$$

47 S.5 Virus durability parameters

48 The persistence terms for virus in the environment (In equation (S1) these are defined as η^k , $k = b, db, a$) are
 49 time-dependent (as they rely on temperature [5]), taking a 'winter' value and a 'summer' value in Delaware Bay
 50 and the breeding grounds for both migrating ducks and ruddy turnstones. They are calculated from weather data
 51 in the different locations [6, 7, 8] and using [5] to convert this to virus persistence in the environment. The virus
 52 persistence at the ruddy turnstones' wintering ground (assuming to be coastal Brazil [9]) is taken to constant
 53 ($\eta^w = 167.9$, superscript w represents wintering ground) throughout the year as the region has very little variation
 54 in temperature [10]. The functions for persistence rate of virus at the duck breeding grounds, Delaware Bay and
 55 the Ruddy Turnstone breeding grounds (denoted by superscripts b, db and t respectively) are

$$\eta^b(t) = \begin{cases} 24.3 & \text{if } 0.33 < t < 0.83, \\ 4.9 & \text{otherwise,} \end{cases} \quad (S15)$$

$$\eta^{db}(t) = \begin{cases} 42.6 & \text{if } 0.33 < t < 0.83, \\ 13.9 & \text{otherwise} \end{cases} \quad (S16)$$

56 and

$$\eta^t(t) = \begin{cases} 10 & \text{if } 0.33 < t < 0.83, \\ 1.6 & \text{otherwise} \end{cases} \quad (\text{S17})$$

57 respectively. Varying these values does not qualitatively affect the results presented in the paper.

58 S.6 Model Analysis: R_0

59 Calculating the effective R_0 value for the system (as described in “The Model” section of the paper), in the
60 absence of seasonality in any of the parameters and using the spectral radius method [11] gives the characteristic
61 polynomial

$$\lambda^4[(R_0^u - \lambda)(R_0^r - \lambda)(R_0^m - \lambda) + (R_0^{rm}R_0^{mr} + R_0^{ru}R_0^{ur})\lambda - (R_0^{rm}R_0^{mr}R_0^u + R_0^{ru}R_0^{ur}R_0^m)] = 0. \quad (\text{S18})$$

62 Here $R_0^i = (\beta^{ii} + \frac{\omega\rho W}{\eta})\frac{S(0)^i}{(\gamma^i + \mu^i)}$ for $i = u, r, m$ ($S(0)^i$ is the initial value of susceptibles for host species i ,
63 $W = \frac{\rho L}{\rho L + \kappa}$ where L is any environmental reservoir of the virus that species i contacts.). For the interactions
64 between two species, $R_0^{ij} = (\beta^{ij} + \frac{\omega\rho V}{\eta^{db}})\frac{S(0)^j}{(\gamma^i + \mu^i)}$ (here the environmental component is limited to Delaware Bay
65 as it is the only location where heterospecific interactions occur in the model).

66 We can solve (S18) in both the presence and absence of heterospecific interactions; in the presence of these
67 and extending the analysis to time-dependent functions for $R_0^i, R_0^i(t)$, (where $R_0^i(t)$ is the R_0^i value if one infected
68 individual enters an entirely susceptible population at time t [12]) we numerically solve (S18) for the effective R_0
69 value [13] (R_0^e); the results are shown in figure 3(a). The absence of heterospecific interactions (but maintaining
70 time-dependent functions as described above) gives R_0^e values for each species:

- 71 1. Effective $R_0^m(t) = (\beta^{mm}(t) + \frac{\omega^{mdb}\rho^{mdb}V(t)}{\eta^{db}(t)} + \frac{\omega^{mb}\rho^{mb}B(t)}{\eta^b(t)})\frac{S^m(t)}{(\gamma^m + \mu^m(t))}$,
- 72 2. Effective $R_0^r(t) = (\beta^{rr}(t) + \frac{\omega^r\rho^rV(t)}{\eta^{db}(t)})\frac{S^r(t)}{(\gamma^r + \mu^r(t))}$,
- 73 3. Effective $R_0^u(t) = (\beta^{uu}(t) + \frac{\omega^{udb}\rho^{udb}V(t)}{\eta^{db}(t)} + \frac{\omega^{ua}\rho^{ua}A(t)}{\eta^a(t)})\frac{S^u(t)}{(\gamma^u + \mu^u)}$.

74 To further consider the role of the various seasonalities present in the model, we also plotted an average
75 effective R_0 value over time (calculated each day as the mean of effective R_0 from that day and the following six
76 days - thus covering the average infectious period for an infected bird). The results are shown in figure S1 and
77 compared with the effective R_0 as shown in figure 3. The results are very similar.

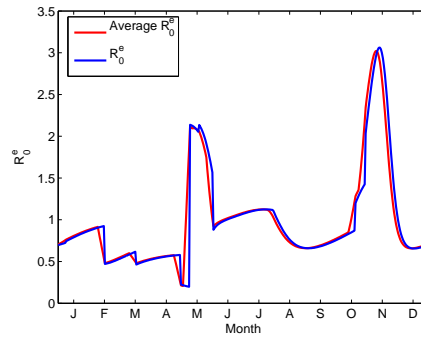


Figure S1: Plot of the average effective R_0 (calculation described in the supporting text) and the effective R_0 as shown in figure 3(a) (with the inclusion of heterospecific interactions).

78 **S.7 Data plots and Parameter Estimation**

79 We show the data plot we use in our parameter estimation. Figure S2(a) shows prevalence in mallard populations
 80 from several different studies (given in [14]) against the prevalence curve for migratory ducks, found from the
 81 model using the parameters estimated below, and figure S2(b) shows the apparent prevalence in American black
 82 ducks as found in Delaware (see [15]) against the prevalence curve for resident ducks from the model, using the
 83 parameters as estimated below. We fitted the model (S11) to available data using sum of squared errors (SSE)
 84 calculated during year 98 of the simulation (year chosen at random). To calculate the SSE, for each time t_i for
 85 which we have data, we assign our model prediction to be y_i and the data point to be x_i . We then sum the
 86 squares of the difference, $(y_i - x_i)^2$, and the minimum value of this sum provides the best fit for the data [16].
 87 We fit both the migratory duck and resident duck prevalence trajectories individually, then normalised the SSE for
 88 each and summed the results. We varied β_0 , β_1 , ϵ and ρ in both duck hosts, assuming that these parameters were
 89 equal in both species. We set the direct transmission rate in ruddy turnstones to be half that of the duck species,
 90 with their amplitude of seasonality in transmission $\beta_1 = 0.5$. We assumed their environmental consumption rate
 91 was equal to that of the ducks and did not vary their duration of immunity. The SSE results for varying β_0 , β_1
 92 and ρ are shown in figure S3(a); we took the best β_0 and β_1 values from this result and then varied ϵ and ρ . The
 93 best SSE (at $\beta_1 = 0.75$) in this case is shown in figure S3(b).

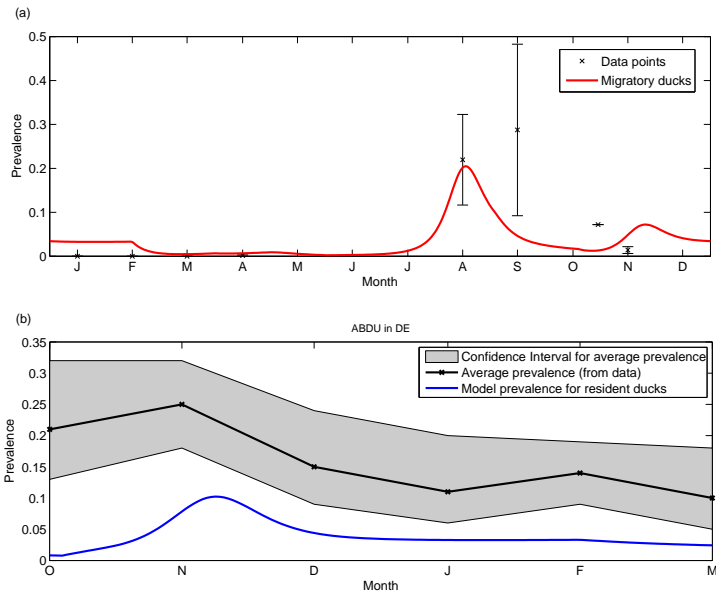


Figure S2: (a) - Prevalence Data for migratory mallards in the USA and Canada against the model fit (red line) for migrating ducks. Data from a number of different sources (taken from Table I in [14]). (b) - Prevalence data collected from American black ducks in Delaware [15] between 2007 and 2010 compared to the model fit for resident ducks.

94 The minimum SSE found was 0.9575 and corresponds to parameter values of $\beta_0 = 0.01$; $\beta_1 = 0.75$; $\rho =$
 95 $1.3804 * 10^{-12}$ and $\epsilon = 2.004$ (duration of immunity ≈ 6 months). The low value of ρ must be considered in
 96 context - ρ is never used in isolation in the model, but always as a multiplier for a virus class. In the standard
 97 model, values for V are of the order of 10^{12} , 10^{13} and so the environmental reservoir is able to contribute
 98 meaningfully to overall virus transmission.

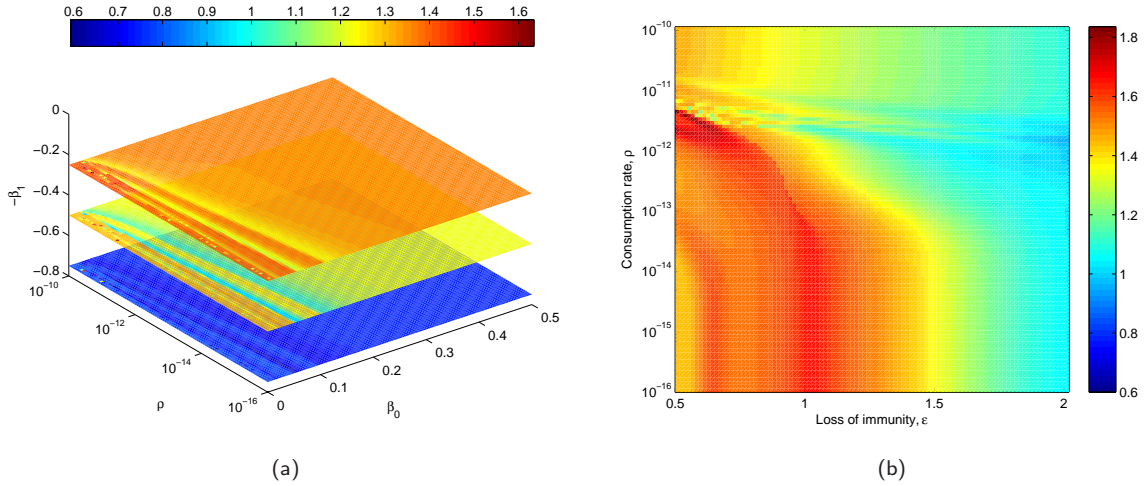


Figure S3: Plots show the sum of square errors (SSE) as various parameters vary. Figure S3(a) shows the SSE as β_1 , β_0 and ρ vary; ρ is plotted on a log scale. Figure S3(b) shows the SSE as ρ and ϵ vary; $\beta_0 = 0.01$ and $\beta_1 = 0.75$.

99 S.8 Sensitivity Analysis

100 We conduct a thorough sensitivity analysis on several of the fixed parameters to assess the robustness of the
 101 qualitative phenomena produced by the model. We first considered the role of the initial conditions; figure S4
 102 shows that there is a single attractor for the parameters in Table 1 and so initial condition choice does not impact
 103 the model dynamics.

104 We considered the impact of many of the infection parameters (direct transmission rate β , consumption rate
 105 ρ , rate of immunity loss ϵ , recovery rate γ and virus persistence η), varying them by 10% in either direction to
 106 create two scenarios - either an increase in transmission potential (i.e. high β , ϵ and ρ ; low η and γ), or a decrease
 107 in transmission potential (i.e. low β , ϵ and ρ ; high η and γ). The results are shown in figure S5 and show that
 108 the results remain qualitatively very similar to the original, although in the case of high transmission potential,
 109 prevalence is non-zero in both duck species throughout the year.

110 Demographic parameters (birth and death rates) were also varied in all species (within 10% of their assigned
 111 values in Table 1); the results are shown in figure S6. We see that these parameters have little impact on the
 112 qualitative dynamics observed in the system.

113 We tested other assumptions in our model to assess their impact. First, we tested our assumption that the
 114 average transmission rate in ruddy turnstones, β_0^u , is half that in ducks, and also tested the effect of the amplitude
 115 of seasonality in the ruddy turnstone direct transmission rate. The results are shown in figure S7 and demonstrate
 116 that peak prevalence will increase as the amplitude of seasonality increases.

117 Varying the consumption rate in ruddy turnstones (to either $\rho = 10^{-14}$ or $\rho = 10^{-13}$) give the results in figure
 118 S9. There is very little qualitative difference between these results and those presented in the main text.

119 We then tested the assumption that inter-species transmission rates are the average of both intra-species
 120 transmission rates; the results are given in figure S8. They show that, in the extreme case of no inter-species
 121 transmission, the dynamics of the system are altered, but that a change in the magnitude of these rates does
 122 not qualitatively affect the dynamics. We tested the inter-species transmission rate between the duck species by
 123 making it both larger and smaller than the value used in the main text; with the interaction between the resident
 124 ducks and ruddy turnstones we only decreased the interaction rate as it is unlikely to grow. However, based on
 125 the result shown below we suspect that the qualitative dynamics of the system would not be affected even in this
 126 case.

127 Figure S10 shows the peak prevalences for both ruddy turnstones and migrating ducks as the number of Ruddy
 128 Turnstones (figure 10(a)) and resident ducks (figure 10(b)) are varied. The dynamics in the system show that

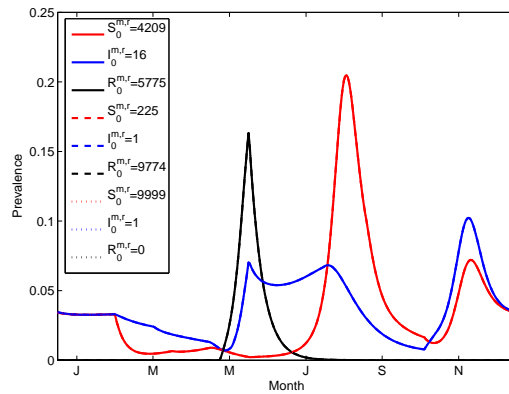


Figure S4: Prevalence curves for all species for varying initial conditions in both duck species. The three choices for initial conditions in the duck species were i) $S_0 = 4209$, $I_0 = 16$, $R_0 = 5775$; ii) $S_0 = 225$, $I_0 = 1$, $R_0 = 9774$; iii) $S_0 = 9999$, $I_0 = 1$, $R_0 = 0$. In all cases the initial conditions for ruddy turnstones were $S_0 = 9999$, $I_0 = 1$ and $R_0 = 0$. For each set of initial conditions, the red curves show the prevalence in migrating ducks, the blue the prevalence in resident ducks and the black the curve in ruddy turnstones.

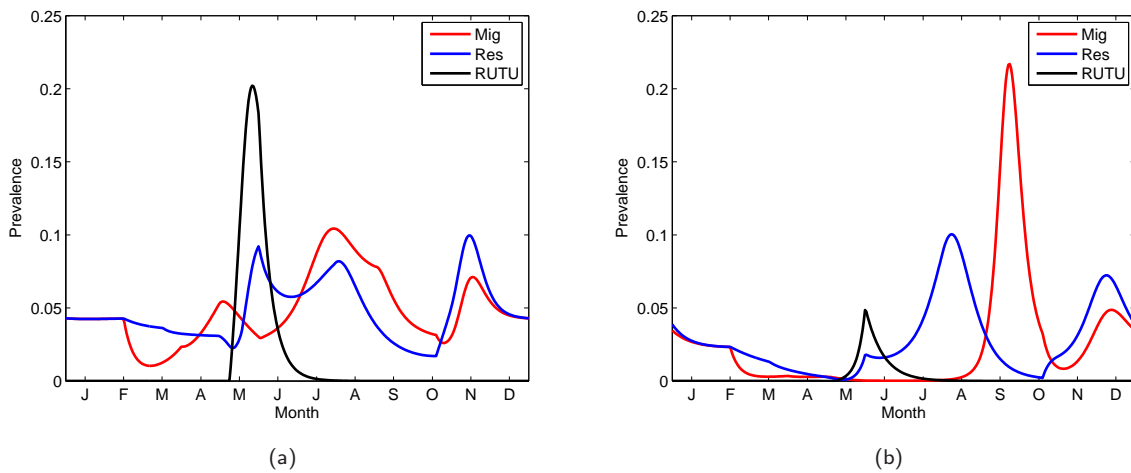


Figure S5: Plots show the model prevalence in all species for either a high transmission potential (S5(a)) or a low transmission potential (S5(b)) (see text for details on transmission potential). Variation in parameters is described in the text.

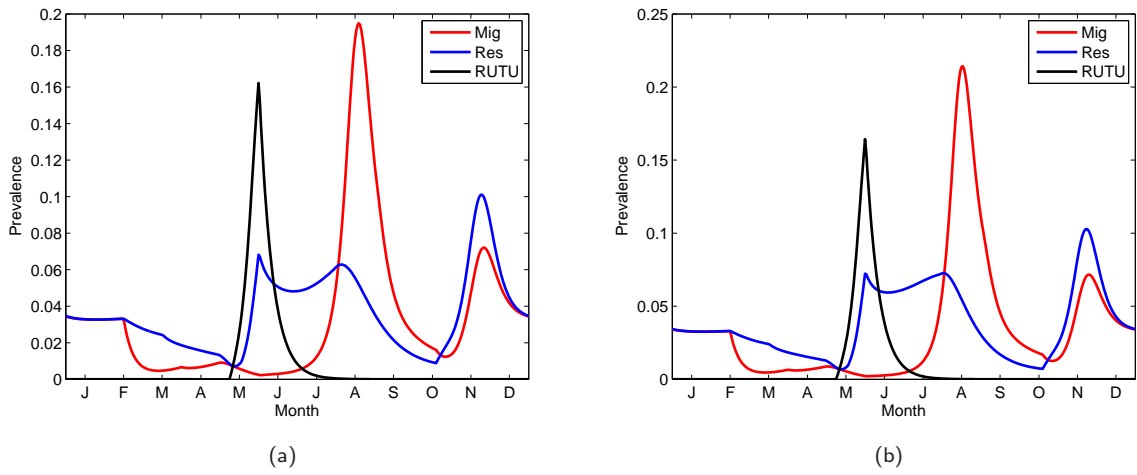


Figure S6: Plots show the model prevalence in all species for either longer-lived (S6(a)) or shorter-lived (S6(b)) birds. Variation in parameters is described in the text.

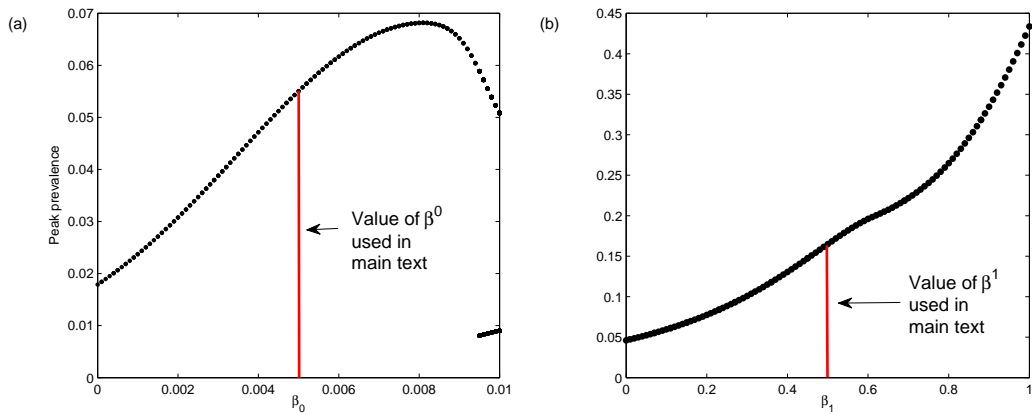


Figure S7: Plots showing the peak prevalence in ruddy turnstones as either their average direct transmission rate (S7(a)) (with no seasonality) or the amplitude of seasonality (with $\beta_0^u = 0.005$) in transmission (S7(b)) varies.

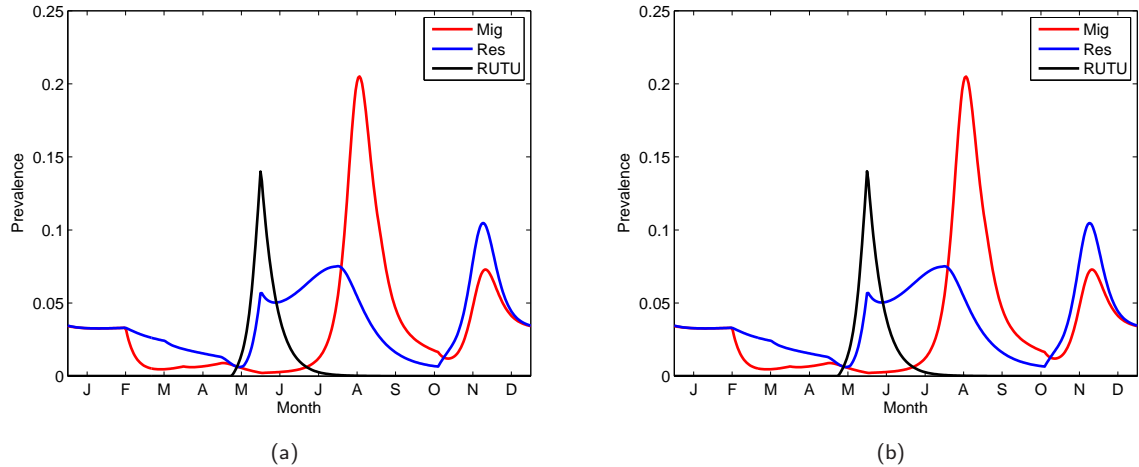


Figure S8: Plots showing model prevalence in all species for consumption rate in the ruddy turnstones of either $\rho = 10^{-14}$ (S8(a)) or $\rho = 10^{-13}$ (S8(b)).

129 migrating duck prevalence is not impacted by the population size of the ruddy turnstones, although this does have
 130 an impact on prevalence in the ruddy turnstones, but that a change in population size in resident ducks could tip
 131 the migrating ducks into a different prevalence curve - this can be seen in figure S12. For ruddy turnstones, an
 132 increase in the resident duck population size will increase their peak prevalence level.

133 We tested the model by removing one or more key elements to assess how it performed. Many of these may
 134 be considered unrealistic in the real-world, but do give an insight into the model behaviour. We first considered
 135 the model with no environmental reservoir, finding that we could no longer observe a peak in prevalence in ruddy
 136 turnstones (figure S11). Removing each of the three species in turn (but assuming the remaining species still
 137 spent time in Delaware Bay) led to the dynamics in figure S12. The removal of the ruddy turnstones led to similar
 138 dynamics appearing in both duck species, with no May peak in the resident duck species. Removing the migrating
 139 ducks delayed the final peak of the year previously seen in resident ducks, with it now occurring at the start of
 140 the year rather than the end. Removing the resident ducks from the model led to a loss of prevalence peaks in
 141 ruddy turnstones and the appearance of an early peak in the migrating ducks. There are no North American data
 142 on which to test this, but similar dynamics have been noted in mallards in Europe [17]. Finally, we left all species
 143 in the model, but prevented both migrating species from travelling through Delaware Bay (figure S13). In this
 144 case we still do not observe a prevalence peak in ruddy turnstones, and the migrating ducks show a prevalence
 145 curve with a post-breeding peak and lower (although non-zero) prevalence for the rest of the year. The resident
 146 ducks show two main peaks in the year, one of which occurs post-breeding.

147 Finally, we relaxed our assumption that the average direct transmission rate in both resident and migratory
 148 ducks was the same (i.e. we assumed that $\beta_0^m = \beta_0^r$). Figure S14 shows the prevalence curves for all three species
 149 in the system, using parameters for duration of immunity (ϵ) and consumption rate (ρ) that were estimated using
 150 the SSE procedure described in Section S.7 (values for the direct transmission rates were set as shown in figure
 151 S14). In all cases, the value of ϵ estimated was the same as for the main text, and the value of ρ varied between
 152 $1.0471 * 10^{-12} < \rho < 1.8197 * 10^{-12}$, depending on the values of β^m and β^r . These results show that relaxing
 153 this assumption 1) makes very little quantitative difference to the estimated values of ϵ and ρ and 2) does not
 154 qualitatively affect the results.

155 S.8.1 Latin Hypercube Sampling

156 We used Latin hypercube sampling to assess the influence of certain key parameters on the definition of Delaware
 157 Bay as an avian influenza 'hotspot' - in particular, we focused on the role of direct transmission in both duck
 158 species (both the average transmission rate β_0 and the amplitude of seasonality β_1); the duration of immunity

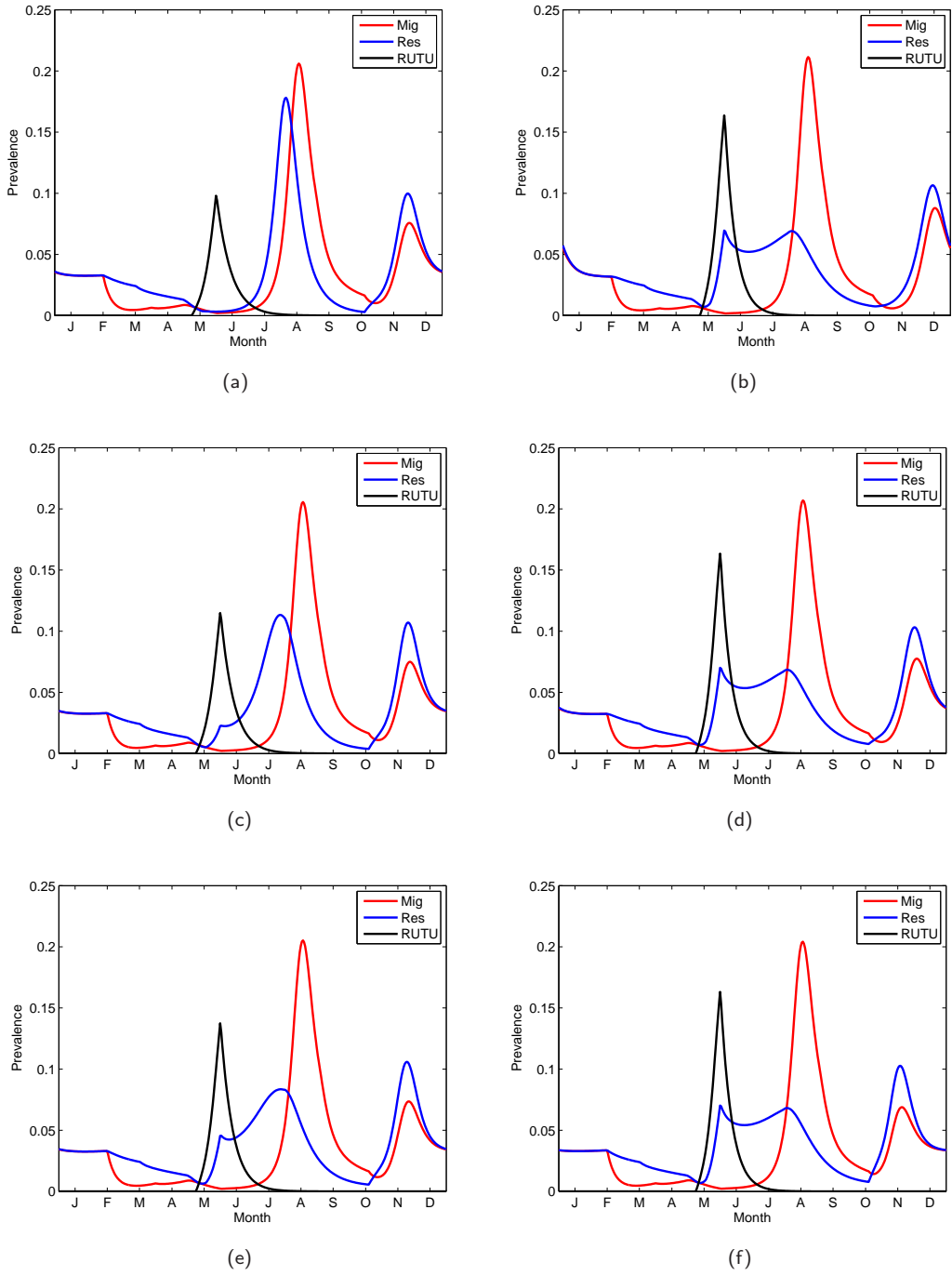


Figure S9: Plots show the model prevalence in all species for varying inter-species transmission rates between either the resident ducks and ruddy turnstones (S9(a),S9(c),S9(e)) or between resident ducks and migratory ducks (S9(b),S9(d),S9(f)). In the first row (S9(a),S9(b)), no inter-species transmission is included; the second row shows less inter-species transmission than in the main text (between resident ducks and ruddy turnstones (S9(c)) it is a third of the value used in the main text; between the resident and migrating ducks (S9(d)) it is half the value used in the main text) and the final row shows the prevalence when inter-species is either $\frac{2}{3}$ the value used in the main text (between resident ducks and ruddy turnstones; S9(e)) or 1.5 times the value used in the main text (between resident and migrating ducks; S9(f)).

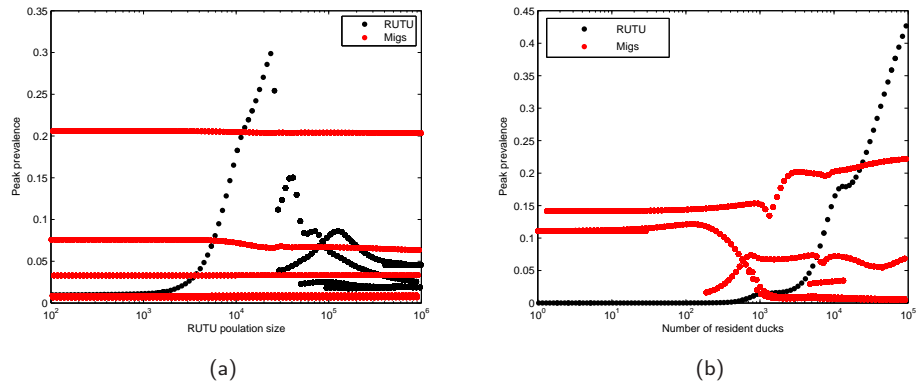


Figure S10: Peak prevalence in ruddy turnstones and migrating ducks as the population size of the Ruddy Turnstones (figure S10(a)) and resident ducks (figure S10(b)) varies. The ruddy turnstone peak prevalence is shown in black and the migrating ducks in red.

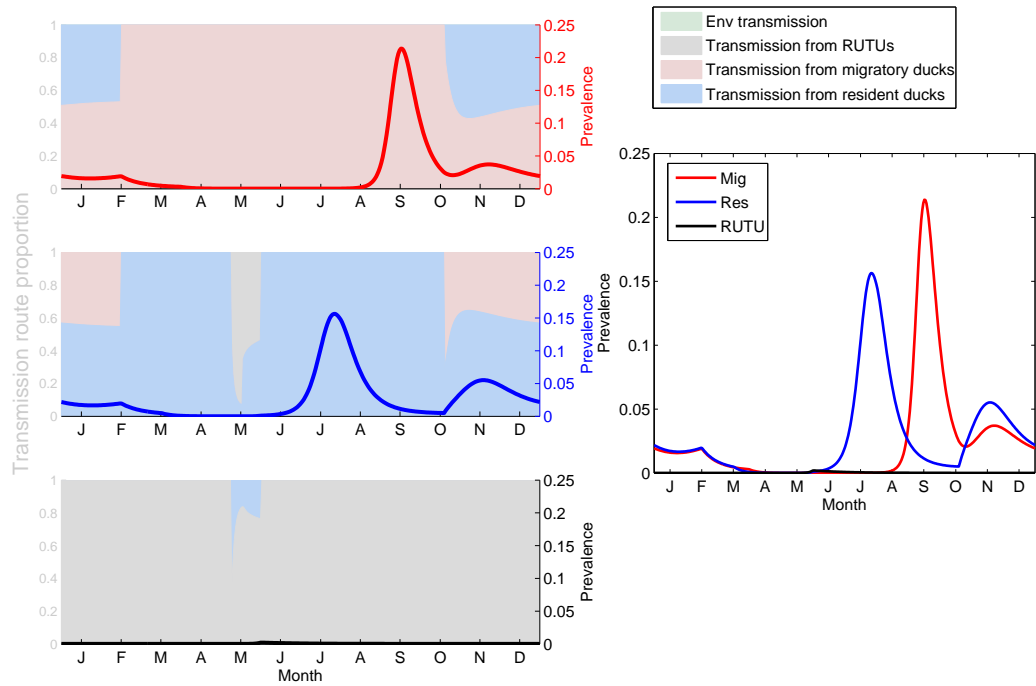
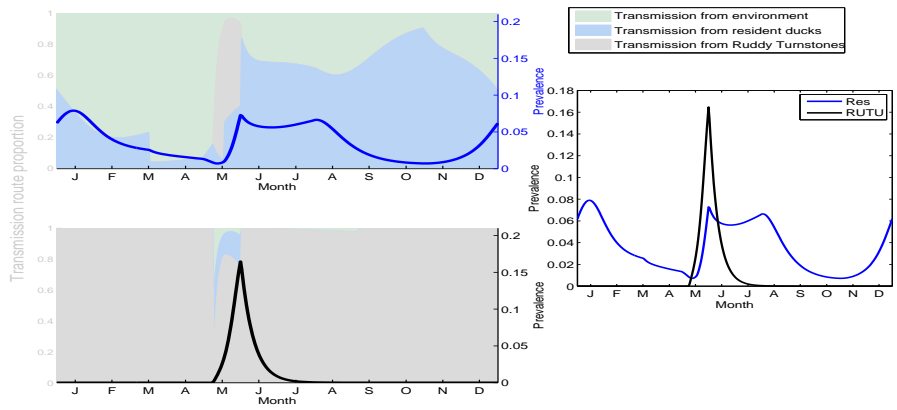
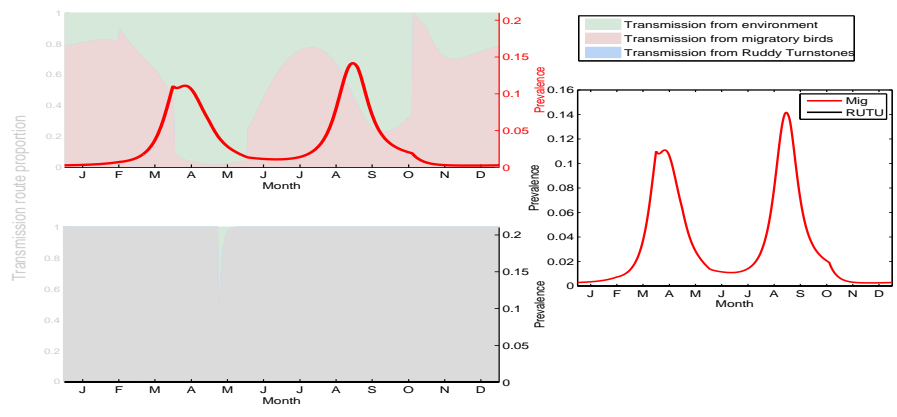


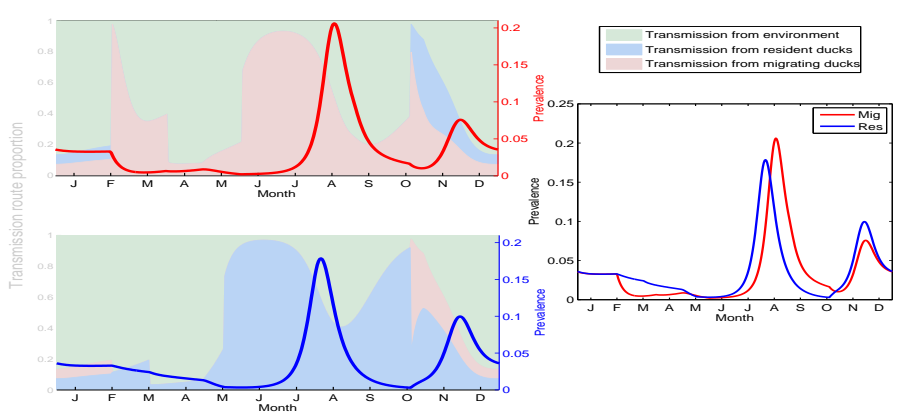
Figure S11: Plots showing the prevalence (right hand figure) and proportion of transmission route when there is no environmental reservoir for the virus.



(a)



(b)



(c)

Figure S12: Plots showing the prevalence (right hand figure) and proportion of transmission route (left hand figures) when one of the species (migrating ducks: S12(a); resident ducks: S12(b); ruddy turnstones: S12(c)) is removed from the model.

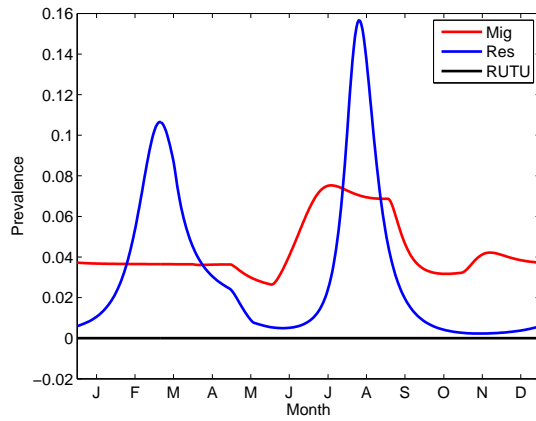


Figure S13: The prevalence curves for all three species when neither migrating species travel through Delaware Bay.

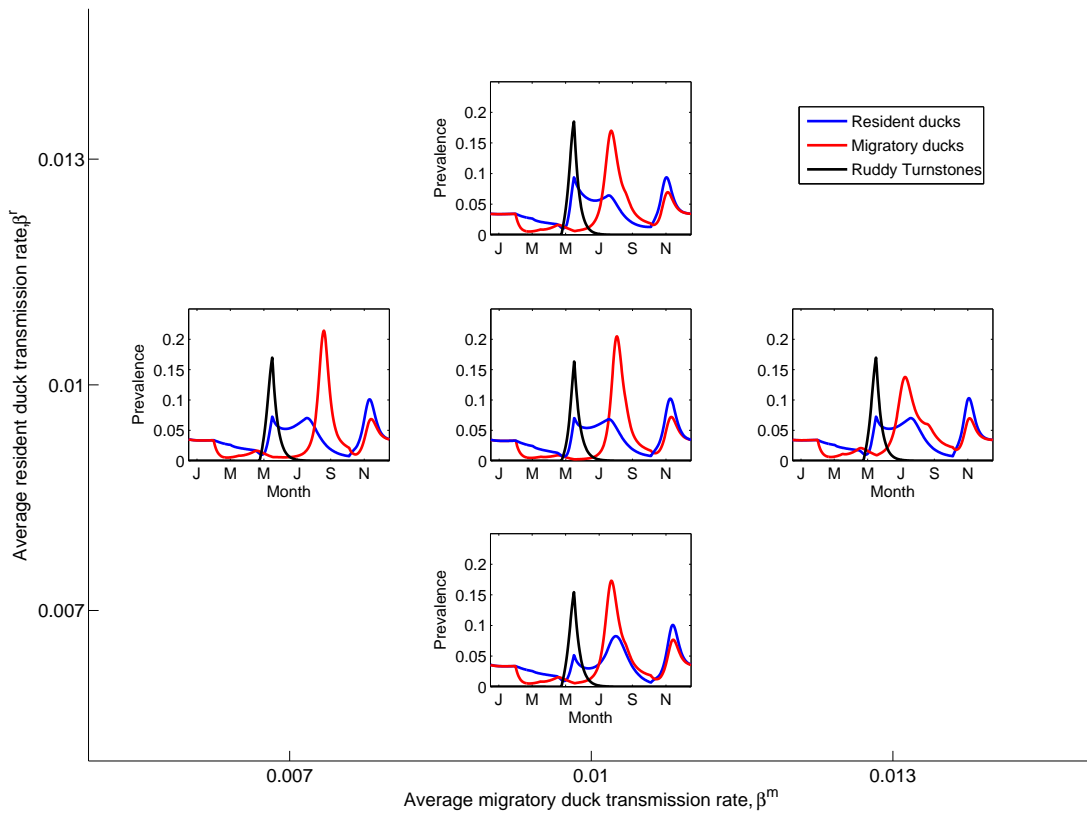


Figure S14: The prevalence curves for all three species using parameters ϵ and ρ estimated using the SSE when the assumption that $\beta_0^m = \beta_0^r$ is relaxed (all other parameters as in Table 1).

159 in both duck species; the recovery rates in all species, the consumption rate in all species and the number of
 160 horseshoe crabs. For each of these parameters, we gave them a range (see table S-1) and created Latin hypercube
 161 samples of 100 sets of 5 of the 6 parameters mentioned - the final parameter (a different one for each sample) was
 162 also varied, but at set intervals throughout its range to create the boxplots seen in figure 4. For each parameter
 163 set, peak prevalence in ruddy turnstones in Delaware Bay was found to determine how it is influenced by different
 164 factors within the model. We also compared these results to the peak prevalence found when only one of the
 165 parameters in question was allowed to vary and all others remained as given in table 1 (shown as a black line in
 166 figure 4).

167 When varying β_0 in the duck species, we did not vary the average direct transmission rate in ruddy turnstones
 168 (β_0^u) - it remained at 0.005 - but the interaction terms between the resident ducks and ruddy turnstones (β_0^{ur}
 169 and β_0^{ru}) were calculated as the average of the two values (β_0^u and β_0^r) used. We further assumed, when varying
 170 β_0 , β_1 and ϵ , that these values were equal in the duck species. Similarly when varying γ and ρ in all hosts, we
 171 assumed that they remained equal across all hosts (as in the standard parameter set from Table 1).

Table S-1: Table of parameter ranges for Latin hypercube sampling.

Parameter	Range
<i>Migrating and Resident ducks</i>	
Average direct transmission, β_0	0-0.05
Amplitude of seasonality, β_1	0-1
Loss of immunity, ϵ	0.5-2.004
<i>All species</i>	
Recovery rate, γ	26-122
Consumption rate, ρ	$10^{-14} - 10^{-10}$
<i>Ruddy turnstones</i>	
Number of horseshoe crabs, E	$1 - 10^8$

172 S.9 Local Lyapunov Exponents

173 LLEs determine, for a given point on the long-term attractor, whether or not a slight perturbation to the system
 174 will result in a diverging trajectory (i.e. whether an invasion event could succeed). The LLE at a given point on
 175 the long-term attractor is equal to the maximum real eigenvalue of the Jacobean matrix for the system at that
 176 point [18]. The sign of the LLE is key to determining whether an invasion event can take place, with a positive
 177 LLE indicating that a perturbation to the system at that point will result in a trajectory that diverges from the
 178 original, unperturbed trajectory. In other words, a positive LLE means that the introduction at that point of
 179 another strain of AIV, which interacts with the dynamics of the the first, will lead to a successful invasion by the
 180 second strain. Conversely, a negative LLE means that a new strain introduced at that point will not be able to
 181 successfully invade [19].

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- 182
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