Overwintering of West Nile Virus and its implications for outbreak risk

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Abstract

West Nile Virus (WNV) is an emerging vector-borne disease in Europe. WNV re-occurs in several European countries despite limited transmission in the winter due to mosquito inactivity. It remains unclear how WNV is able to overwinter, although vertical transmission in mosquitoes, direct avian transmission and reintroduction through migratory birds are considered. In spring, conditions are still unfavourable to ensure sustained WNV transmission from host to vector and vector to host, so WNV has to persist in this period. This research looked how efficient WNV can overwinter and how well WNV can persist in the spring when transmission conditions are still unfavourable. A scoping literature review was performed to find information on vertical transmission in mosquitoes, WNV persistence in birds, reintroduction through migration, and direct avian transmission of WNV. A stochastic transmission model was used to study how the probability and size of a WNV outbreak in summer is affected by initial WNV prevalence in mosquitoes and birds, the temperature in spring, and the moment the mosquito season starts. Vertical transmission and WNV persistence are well-studied showing low efficiency of vertical transmission and long WNV persistence in birds. Reintroduction through migration and direct avian transmission are not fully understood yet, although evidence suggests effective direct avian transmission. Initial mosquito prevalence was found to have a large impact on the probability and size of a WNV outbreak with low prevalences already resulting in a high chance of an outbreak with a high final size of infected birds at the end of the transmission season. Higher spring temperatures were found to impact the probability of a WNV outbreak, which was also of a larger size . Avian prevalence and initial conditions were not found to have a large impact on the probability and size of a WNV outbreak. Since prevalence in the mosquito population was found to have a major impact on the probability and risk of an outbreak it would be useful if more research will be performed on the mosquito dynamics at lower temperatures. Especially mosquito mortality at low temperatures is still not fully understood, but this seemed to have a major impact on the probability and size of a WNV outbreak.

Introduction

West Nile Virus (WNV) is a vector-borne disease from the family of *Flaviviridae* originating from Sub-Saharan Africa. WNV has been introduced to and spread within the United States of America (CDC, 1999; Hadfield et al., 2019) and Europe (Bakonyi et al., 2006; ECDC, 2023) in the last 25 years. Although humans are considered to be dead-end host, WNV could still lead to symptomatic infections and illness in humans (Zou et al., 2010). The transmission cycle of WNV consists of mosquitoes as vectors and birds as hosts. Different mosquito species are known to transmit WNV, but *Culex pipiens* is found to be the most competent vector of WNV (Vogels et al., 2017). *Cx. pipiens* species consists of two biotypes: *pipiens* and *molestus*. From these biotypes *pipiens* is the more competent WNV vector since it is ornithophilic regarding its blood feeding preference (Koenraadt et al., 2019). Adult females of *Cx. pipiens pipiens* are known to go into diapause during winter due to unfavourable environmental conditions (Koenraadt et al., 2019; Reisen & Wheeler, 2019). This inactivity of mosquitoes results in an inhibition of sustained WNV transmission in winter.

Despite this inactivity of mosquitoes, WNV often re-occurs in the following transmission season, indicating that the virus is able to persist during winter. This means that WNV has to use overwintering mechanisms to survive. Several overwintering mechanisms have been shown to be possible (Reisen et al., 2006; Reisen & Wheeler, 2019). These mechanisms include continued transmission at lower rates, vertical transmission within mosquitoes and persistence of WNV in hosts. Furthermore, WNV could be reintroduced after the winter by migrating birds (García-Carrasco et al., 2023; Swetnam et al., 2018). Although these overwintering mechanisms are known it is still unclear how efficient these mechanisms are and to what extent they contribute to overwintering of WNV.

After surviving the winter, WNV transmission is still limited when the adult female mosquitoes emerge from their diapause in spring (Koenraadt et al., 2019; Reisen & Wheeler, 2019). However, WNV transmission increases towards the summer (Ziegler et al., 2019). This change in WNV transmission efficiency is induced by changing environmental factors (Giesen et al., 2023). Especially temperature plays a crucial role in WNV. Higher temperatures in the summer increase WNV transmission. Research has shown that temperature can influence vector abundance (Ewing et al., 2016; Groen et al., 2017), biting frequency (Ruybal et al., 2016;Di Pol et al., 2022) and the extrinsic incubation period (EIP) (Di Pol et al., 2022).

These elements all influence WNV transmission by their effect on the basic reproduction number (R_0). The value of R_0 indicates how many birds and mosquitoes are on average infected by an infectious bird or mosquito in a susceptible population. If R_0 is higher than 1, the infectious disease has met the conditions to develop into a major outbreak. On the other hand, only minor outbreaks of the infectious disease can occur when R_0 is lower than 1. Minor outbreaks have significantly lower numbers of infected cases and the duration of the outbreak is shorter compared to major outbreaks (Tritch & Allen, 2018). A minor outbreak will eventually always die out making them unfavourable for infectious diseases. A mathematical model can be used to understand and quantify the dynamics and characteristics of an infectious disease under changing conditions, for example temperature. In the case of WNV, several models have been developed that explain temperature-dependent WNV transmission (Bhowmick et al., 2020; Kioutsioukis & Stilianakis, 2019; Laperriere et al., 2011) or the closely related Usutu virus (Rubel et al., 2008). Furthermore, research has been performed that showed how spring temperatures are related to human cases of WNV (Marini et al., 2019). Other research has looked how WNV could sustain within a bird population during winter (Montecino-Latorre et al., 2018). However, no research has been done that look how spring conditions in temperate regions impact the probability and size of a WNV outbreak.

This research will focus on the persistence of WNV in temperate regions before it can be transmitted efficiently during the summer. It is important to understand how efficiently WNV can persist during unfavourable transmission conditions in order to understand the risk of major outbreaks of WNV in the summer in temperate regions. WNV has a certain probability that it persists till the summer. However, it is still unknown how this chance is dependent on different aspects such as prevalence in the population and the temperature. This requires an understanding of the overwintering efficiency of WNV, as well as an indication of the likelihood that WNV can persist during the spring period with unfavourable conditions for transmission. To understand overwintering efficiency, research will be done on the contribution of the mentioned overwintering mechanisms on the WNV prevalence at the start of the transmission period the next year. Understanding the contribution of these mechanisms provides information on which characteristics are favourable for virus overwintering and how control measures could reduce the overwintering risk. To quantify the likelihood of major WNV outbreaks in the summer, a temperature-dependent stochastic model is developed. This model could help to better understand how efficiently WNV is able to persist in the mosquito and bird populations when temperature is unfavourable for transmission, thereby giving insight in the risk of major WNV outbreaks during the summer.

Materials and Methods

Overwintering mechanisms

A scoping literature search was performed focusing on the efficiency of four overwintering mechanisms of WNV: 1) vertical transmission in mosquitoes, 2) WNV persistence in birds, 3) reintroduction through migratory birds and 4) direct avian transmission. All four were investigated based on the PRISMA method for scoping reviews (Tricco et al., 2018). The PRISMA flow diagrams for the four different methods are given in the Appendix S1-S4 . Publications were found using the PubMed Database. Additional papers were found through snowballing in other papers.

Model

The model to represent WNV transmission is a SEIR-SEI model with birds as hosts and mosquitoes as vectors, see Figure 1. The species *Culex pipiens* was used as vector, since *Culex* species are known to be competent vectors of WNV (Vogels et al., 2017) and the species *Culex pipiens* is commonly present within the Netherlands (Ibañez-Justicia et al., 2015). The common blackbird (*Turdus merula*) was used as a representation of the host species, because the common blackbird is a competent host of WNV (Rubel et al., 2008) and this bird species is commonly present in the Netherlands (Sovon, 2023). The bird and mosquito populations are not constant over time, hence there is a birth (α) and death rate (μ) for both birds and mosquitoes. The mosquito population in the model represents only female adult mosquitoes. The mosquitoes cannot recover from WNV, since their lifespan is too short to recover after infection. The bird population contain individuals from all ages and sex. Birds can recover from WNV (recovery rate γ_B) and experience an additional death rate due to WNV infection (λ_B). The birds that died due to WNV infection are counted in the D_B-compartment (Figure 1). The model assumed that there is only transmission between mosquito and birds and no vertical transmission or horizontal transmission between birds. The model simulated the transmission of WNV in this host-vector system during the period April-August.

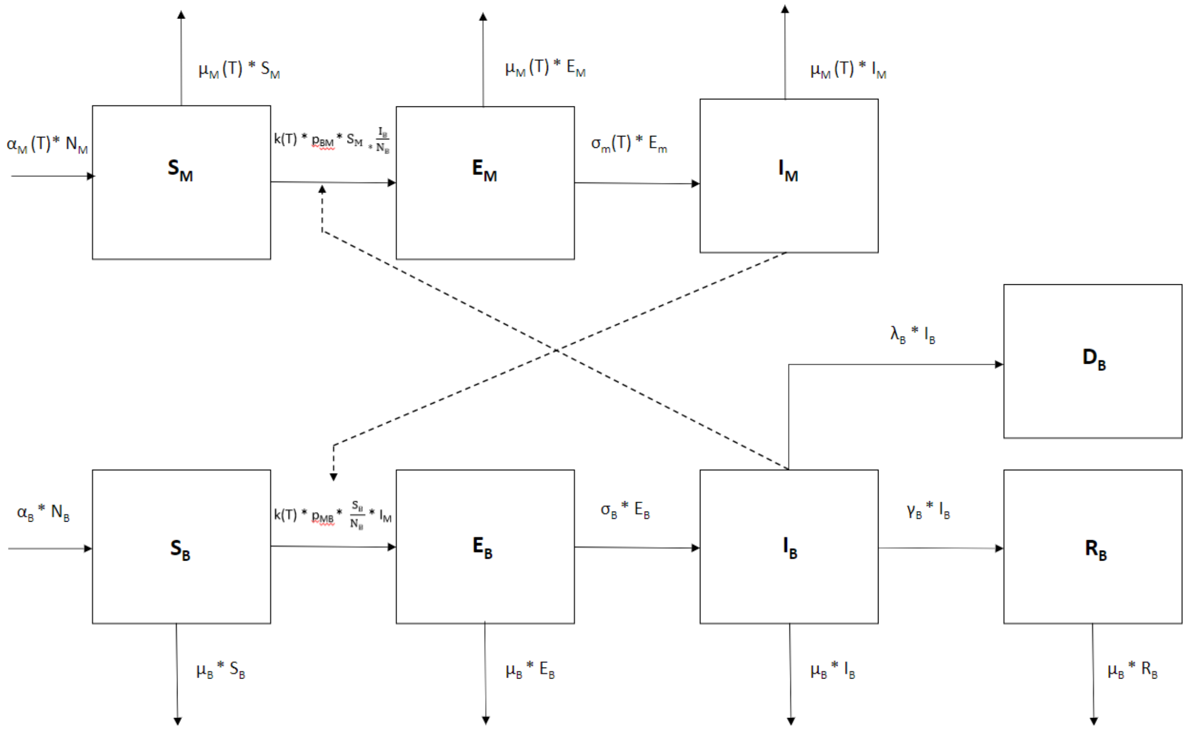

Figure 1. The SEIR model for WNV infection. T indicates that a parameter is temperature-dependent

The model described above and shown in Figure 1 consists of 8 ordinary differential equations (ODEs). The 3 ODEs for the mosquito population are:

$$\frac{dSm}{dt} = \alpha_M(T) * N_M - \mu_M(T) * S_M - k(T) * p_{BM} * S_M * \frac{I_B}{N_B} \tag{1}$$

$$\frac{dEm}{dt} = k(T) * p_{BM} * S_M * \frac{I_B}{N_B} - \mu_M(T) * E_M - \sigma_M(T) * E_M \tag{2}$$

$$\frac{dIm}{dt} = \sigma_M(T) * E_M - \mu_M(T) * I_M \tag{3}$$

The 5 ODEs for the bird population are:

$$\frac{dSb}{dt} = \alpha_B * N_B - \mu_B * S_B - k(T) * p_{MB} * I_M * \frac{S_B}{N_B} \tag{4}$$

$$\frac{dEb}{dt} = k(T) * p_{MB} * I_M * \frac{S_B}{N_B} - \mu_B * E_B - \sigma_B * E_B \tag{5}$$

$$\frac{dIb}{dt} = \sigma_B * E_B - \mu_B * I_B - \gamma_B * I_B - \lambda_B * I_B \tag{6}$$

$$\frac{dRb}{dt} = \gamma_B * I_B - \mu_B * R_B \tag{7}$$

$$\frac{dDb}{dt} = \lambda_B * I_B \tag{8}$$

The definitions of the parameters can be found in Table 3. The equations 1-8 can be solved numerically when given estimates for the parameters and the initial conditions. The capital T between brackets (T) indicates that a parameter is temperature-dependent.

Temperature data

As discussed before, the mosquito population dynamics and the transmission of WNV is influenced by the temperature. The mean daily temperature within the Netherlands was used to calculate the value of temperature-dependent parameters in the model. This data was retrieved from the KNMI (KNMI, 2023a) for the years 2013-2022. For the months May until August temperature values from 2022 were used in all simulations. To assess the impact of variation in early spring temperature, April temperatures of the years 2018 and 2021 were used to study the effect of respectively a warm and cold April on the probability of a summer WNV outbreak, see Table 1. Temperature data from March 2022 was used to study the effect of an earlier onset of the mosquito season on the probability of a summer WNV outbreak.

Table 1. Average April temperatures for the years 2013-2022 and the difference with the average temperature for the whole period

Year	mean temperature in April (°C)	Difference compared to mean April temperature in period 2013-2022 (°C)
2022	9.3	-0.37
2021	6.7	-2.97
2020	11.1	1.43
2019	10.9	1.23
2018	12.2	2.53
2017	8.6	-1.07
2016	8.7	-0.97
2015	9.0	-0.67
2014	12.1	2.43
2013	8.1	-1.57
Period 2013 - 2022	9.67	0

Model parameters

Model parameters were taken from literature. However, most models with an explicit mosquito population used an additional larval compartment in the model with a temperature-dependent larval birth and death rate. Since a Gillespie algorithm was used to simulate the model, such an additional larval compartment in the model would result in a lot of events used for the birth and death of larvae. To prevent this computational inefficiency a direct adult birth rate was estimated using a discrete mosquito population model with temperature-dependent parameters taken from literature.

Adult mosquito birth rate

A deterministic model was used to estimate the adult birth rate of mosquitoes. The model consists of two compartments: the larval (L) compartment and the adult (A) compartment. Each compartment has a birth rate or death rate. All parameters are temperature dependent indicated with a (T) in the ODEs. The 2 ODEs in this model are:

$$\frac{dL}{dt} = \alpha_L(T) * A - \mu_L(T) * L - \alpha_A(T) * L \qquad (1)$$

$$\frac{dA}{dt} = \alpha_A(T) * L - \mu_A(T) * A \qquad (2)$$

The parameters in this model were taken from literature, see Table 2. The larval birth rate (α_L(T)) was estimated to be the reciprocal of the gonotrophic cycle (Shocket et al., 2020; Li et al., 2017; Ruybal et al., 2016; Madder et al., 1983; Tekle et al., 1960).

The larval mortality rate (μ_L(T)) was estimated from the following function:

$$\mu_L(T) = \frac{Ln(1-P_{LA}(T))}{-t(T)}$$

With P_{LA}(T) as the larval-to-adult survival probability and t(T) as the larval period. P_{LA}(T) was taken as a temperature function from literature (Shocket et al, 2020; Ruybal et al., 2016; Ciota et al., 2014; Loetti et al., 2011; Mpho et al., 2002a; Mpho et al., 2002b; Madder et al, 1983; Tekle et al., 1960). The larval period was estimated to be the reciprocal of the mosquito development rate (MDR) (Shocket et al, 2020; Ruybal et al., 2016; Ciota et al., 2014; Loetti et al., 2011; Mpho et al., 2002a; Mpho et al., 2002b; Madder et al, 1983; Tekle et al., 1960).

The adult emerge rate ($\alpha_M(T)$) was estimated as the MDR as described above. The adult mortality rate ($\mu_M(T)$) was estimated as the reciprocal of the adult lifespan (Shocket et al., 2020; Ruybal et al., 2016, Andreadis et al., 2014; Ciotta et al., 2014). Due to a lack of data at lower temperatures, at temperatures below 15 ^{0}C the adult mortality rate was estimated as equal to the adult mortality rate at 15 ^{0}C. (Shocket et al., 2020).

Table 2. Emerge and mortality rates of *Culex pipiens* larvae and adults as a function of temperature (T) in ^{0}C

Parameter	**Symbol**	**Function**	**Source**
Larval emerge rate (day^{-1})	$\alpha_L(T)$	f(T) = $1.70 * 10^{-4} * (T - 9.4) * \sqrt{39.6 - T}$	Appendix S5
Larval-to-Adult survival probability	pLA(T)	f(T) = $3.60 * 10^{-3} * (T - 7.8) * (T - 38.4)$	Appendix S5
Larval time (day)	t(T)	f(T) = $\frac{1}{3.76 * 10^{-5} * T * (T - 7.8) * \sqrt{38.5 - T}}$	Appendix S5
Larval mortality rate (day^{-1})	$\mu_L(T)$	f(T) = $\frac{Ln(1 - pLA(T))}{-t(T)}$	Appendix S5
Adult emerge rate (day^{-1})	$\alpha_A(T)$	f(T) = $3.76 * 10^{-5} * T * (T - 7.8) * \sqrt{38.5 - T}$	Appendix S5
Adult mortality rate (day^{-1})	$\mu_A(T)$	for T ≥ 15 °C: f(T) = $\frac{1}{-4.86 * T + 169.8}$ for T ≤ 15 °C: f(T) = 0.0103	Appendix S5

With these outcomes and the known adult mortality rate a direct adult birth rate was estimated using the following function:

$$\alpha_M(T) = \frac{A(t) - A(t-1)}{A(t)} - \mu_A(T)$$

The values of $\alpha_M(T)$ for each day can be found in Appendix S6.

Other mosquito parameters

The model parameters used in the transmission model are presented in Table 3. The mosquito parameters consist of both temperature-dependent functions and temperature-independent values. The emergence rate ($\alpha_M(T)$), death rate ($\mu_M(T)$), biting rate ($k_M(T)$) and the reciprocal of the extrinsic incubation period ($\sigma_M(T)$) are all temperature-dependent, whereas the probability of WNV transmission from both mosquito-to-bird and bird-to-mosquito is temperature-independent.

WNV transmission is dependent on the biting frequency (k(T)) and the probability of transmission either from vector to host (p_{MB}) or host to vector (p_{BM}). As stated before, the biting frequency is equal to the reciprocal of the gonotrophic cycle. Therefore, its value is the same as the larval emerge rate stated in Table 1. The transmission probabilities were estimated by Wonham et al. (2004) to lay between 0.8 and 1 for p_{MB} and between 0.02 and 0.24 for p_{BM}. In this model the values of p_{MB} and p_{BM} were estimated as 1 and 0.125, respectively. The rate at which WNV infected mosquitoes become infectious ($\sigma_M(T)$) is the reciprocal of the EIP (Shocket et al., 2020; Kilpatrick et al., 2008; Dohm et al. , 2002).

Table 3. Parameter values of the model. All functions are functions of temperature (T) in °C

Parameter	**Symbol**	**Value/Function**	**References**
Mosquito emergence rate (day^{-1})	$\alpha_M(T)$	If $\alpha_M(T) < \mu_M(T)$: $\alpha_M(T) = \mu_M(T)$ If $\alpha_M(T) \geq \mu_M(T)$: See Appendix S6	Appendix S5
Mosquito death rate (day^{-1})	$\mu_M(T)$	for T > 15: f(T) = $\frac{1}{-4.86*T+169.8}$ for T ≤ 15: f(T) = 0.0103	Appendix S5
Biting rate (day^{-1})	k(T)	f(T) = $1.70 * 10^{-4} * (T-9.4) * \sqrt{39.6-T}$	Appendix S5
Transmission probability mosquito-to-bird	p_{MB}	1	Appendix S5
Transmission probability bird-to-mosquito	p_{BM}	0.125	Appendix S5
Mosquito transition rate from infected to infectious (day^{-1})	$\sigma_M(T)$	f(T) = $7.38 * 10^{-5} * T * (T-11.4) * \sqrt{45.2-T}$	Appendix S5
Bird birth rate (day^{-1})	α_B	0.0019	Appendix S5
Bird death rate (day^{-1})	μ_B	0.0013	Appendix S5
Bird transition rate from infected to infectious (day^{-1})	σ_B	0.667	Appendix S5
Bird recovery rate (day^{-1})	γ_B	0.1274	Appendix S5
WNV death rate for birds (day^{-1})	λ_B	0.0546	Appendix S5

Bird parameters

The bird death rate of the common blackbird was based on data from Sovon (2023). It was assumed that the bird population remains constant between years but fluctuates within the year due to seasonality of births. The birth period of the blackbird is from April till July (Sovon, 2023). Therefore, the bird birth rate was estimated in such a way that the bird population would almost remain equal during the simulated period if no WNV was present and that the birth rate is zero after 31st of July.

The rate at which infected birds become infectious (σ_B) is the reciprocal of the intrinsic incubation period (IIB). The IIB was estimated as 1.5 days (Rubel et al., 2008).

In contrast to mosquitoes, birds can recover from WNV or can die due to WNV in this model. This results in a recovery rate (γ_B) and a WNV mortality rate (λ_B). The recovery rate of the common blackbird was estimated to be 0.1274 day^{-1} (Rubel et al., 2008; Weisenböck et al., 2002). The WNV mortality rate was estimated to be 0.0546 day^{-1} (Rubel et al., 2008; Weisenböck et al., 2002).

Basic reproduction number

The R_0 was determined from the next generation matrix (NGM). Since the model only contained cross-infection, there was no WNV infection from mosquito-to-mosquito or from bird-to-bird, hence these values were equal to zero. Since R_0 was estimated on the disease-free equilibrium it was assumed that the susceptible population is equal to the total population and that the infected population is very small. The infection from bird to mosquito depends on:

- the bird-to-mosquito transmission (k(T) * p_{BM})
- the probability that an infected mosquito becomes infectious ($\frac{\sigma_M(T)}{\mu_M(T)+\sigma_M(T)}$)
- the time that an infected bird remains infected ($\frac{1}{\mu_B+\gamma_B+\lambda_B}$)
- the vector-host ratio ($\frac{N_M}{N_B}$)

The infection mosquito to bird depends on:

- the mosquito-to-bird transmission ($k(T) * p_{MB}$)
- the probability that an infected bird becomes infectious ($\frac{\sigma_B}{\mu_B + \sigma_B}$)
- the time that an infected mosquito remains infected ($\frac{1}{\mu_M(T)}$)

Given all these values the NGM will become:

$$\begin{array}{cc} & \begin{matrix} M & & & B \end{matrix} \\ \begin{matrix} M \\ \\ B \end{matrix} & \begin{pmatrix} 0 & \frac{N_M}{N_B} * \frac{k(T) * p_{BM} * \sigma_M(T)}{\left(\mu_M(T) + \sigma_M(T)\right) * (\mu_B + \gamma_B + \lambda_B)} \\ \frac{k(T) * p_{MB} * \sigma_B}{(\mu_B + \sigma_B) * \mu_M(T)} & 0 \end{pmatrix} \end{array}$$

The R_0 is the dominant eigenvalue of this NGM:

$$R_0 = \sqrt{\frac{k(T) * p_{MB} * \sigma_B}{(\mu_B + \sigma_B) * \mu_M(T)}) * \frac{N_M}{N_B} * \frac{k(T) * p_{BM} * \sigma_M(T)}{\left(\mu_M(T) + \sigma_M(T)\right) * (\mu_B + \gamma_B + \lambda_B)})}$$

Model initialization

The model was implemented and numerically solved in R (R core team, 2022). A Gillespie algorithm was used to solve the equations of the model. This algorithm was based on section 8.2 from *Epidemics: Models and Data using R* of Ottar Bjornstad (2022). A SEIR model consists of multiple events that can occur (death, birth, infection etc.). The waiting time between two events occurring in a SEIR model is exponentially distributed. The mean waiting time of this exponential distribution is the sum of the rates of all possible events, since it is expected that the system changes with this sum. The Gillespie algorithm first decides a random waiting time from the distribution with the sum of all rates as the mean. This time is used to change the continuous clock. Next, the algorithm chooses which event occurs. The probability that an event occurs is determined by the relative contribution of the rate of each event to the sum of all rates. From all these probabilities one event is picked to occur in the waiting time. Since temperature influences vector birth, mortality, biting frequency and the EIP, the rates of the related events will change over time. This means that both the sum of all rates as well as the relative contribution of each rate to this sum will change over time.

A τ-leap algorithm was used to shorten simulation time. The τ-leap algorithm is based on the Gillespie algorithm described above, but only updates the compartments in the model after a specified time dT has passed, thereby improving the efficiency of the calculations.

Initial mosquito and bird population conditions needed to be specified before the simulation. The initial population conditions needed to give a realistic R_0 with enough individual mosquitoes to use low initial mosquito prevalences for WNV in the mosquito population. Through trial-and-error and initial population was chosen with $N_{mosquitoes} = 30000$ and $N_{birds} = 1200$.

Model outcomes

Four variables were tested for their effect on the probability and size of a WNV outbreak in the summer: initial WNV prevalence in mosquitoes, initial WNV prevalence in birds, temperature in April and timing of the start of the mosquito season (Table 4). The scenarios of initial WNV prevalence in mosquitoes were divided in five different values (range 0 – 0.02%) and the scenarios of initial WNV prevalence in birds (range 0 – 10%), April temperature (normal, cold and warm) and start of the mosquito season (start of April, mid-March and start of March) in three different values. In total 35 different scenarios were investigated. Each scenario was simulated 500 times.

Scenarios were compared using several outcome measures. The size of the WNV outbreak in the summer was determined at the end of August, since afterwards mosquitoes will start to go into diapause which was not taken into account in this model. Probabilities were calculated on the moment R_0 remained above one for the rest of the simulation time. This moment was found to occur at the start of May. Probabilities and final sizes of both mosquitoes and birds were plotted against the initial WNV prevalence of mosquitoes with different plots to compare initial WNV prevalence of birds, April temperatures and start of the transmission season.

Table 4. Overview of all scenarios with the different variables that are studied for their effect on the probability and size of a WNV outbreak in the summer

Scenario	Initial WNV prevalence in mosquito population (%)	Initial WNV prevalence in bird population (%)	April temperature (year)	Start of mosquito season
1	0	0	Normal (2022)	Start April
2	0.0033	0	Normal (2022)	Start April
3	0.0067	0	Normal (2022)	Start April
4	0.0133	0	Normal (2022)	Start April
5	0.02	0	Normal (2022)	Start April
6	0	5	Normal (2022)	Start April
7	0.0033	5	Normal (2022)	Start April
8	0.0067	5	Normal (2022)	Start April
9	0.0133	5	Normal (2022)	Start April
10	0.02	5	Normal (2022)	Start April
11	0	10	Normal (2022)	Start April
12	0.0033	10	Normal (2022)	Start April
13	0.0067	10	Normal (2022)	Start April
14	0.0133	10	Normal (2022)	Start April
15	0.02	10	Normal (2022)	Start April
16	0	0	Cold (2021)	Start April
17	0.0033	0	Cold (2021)	Start April
18	0.0067	0	Cold (2021)	Start April
19	0.0133	0	Cold (2021)	Start April
20	0.02	0	Cold (2021)	Start April
21	0	0	Warm (2018)	Start April
22	0.0033	0	Warm (2018)	Start April
23	0.0067	0	Warm (2018)	Start April
24	0.0133	0	Warm (2018)	Start April
25	0.02	0	Warm (2018)	Start April
26	0	0	Warm (2018)	Mid-March
27	0.0033	0	Warm (2018)	Mid-March
28	0.0067	0	Warm (2018)	Mid-March
29	0.0133	0	Warm (2018)	Mid-March
30	0.02	0	Warm (2018)	Mid-March
31	0	0	Warm (2018)	Start March
32	0.0033	0	Warm (2018)	Start March
33	0.0067	0	Warm (2018)	Start March
34	0.0133	0	Warm (2018)	Start March
35	0.02	0	Warm (2018)	Start March

Results

Literature review

Vertical transmission

Vertical transmission of WNV in mosquitoes was investigated in 21 articles , see Supplementary files Tab S1. The articles contained 26 different species of which eight from the *Culex* genus. *Culex pipiens* was the most studied mosquito species (Anderson et al., 2006, 2008; Blom et al., 2023; Calzolari et al., 2013; Dinu et al., 2015; Dohm et al., 2002; Farajollahi et al., 2005; Fechter-Leggett et al., 2012; Fortuna et al., 2015; Goddard et al., 2003; Micieli et al., 2013; Nelms et al., 2013a; Nelms et al., 2013b; Reisen et al., 2006). Ten articles researched experimental vertical transmission (Anderson et al., 2008, 2012; Baqar et al., 1993; Calzolari et al., 2013; Dohm et al., 2002; Eastwood et al., 2011; Fortuna et al., 2015; Goddard et al., 2003; Micieli et al., 2013; Nelms et al., 2013b), ten articles investigated vertical transmission in the field (Anderson, 2006; Blom et al., 2023; Dinu, 2015; Farajollahi et al., 2005; Fechter-Leggett et al., 2012; Phillips et al., 2006; Reisen et al., 2006; Sudeep et al., 2015; Unlu et al., 2010; Zuñiga et al., 2023)and one article discussed both types of research (Nelms et al., 2013a). In total, 81% (17/21) of the articles found evidence of vertical WNV transmission in mosquitoes (Anderson et al., 2006, 2008, 2012; Baqar et al., 1993; Calzolari et al., 2013; Dinu, 2015; Dohm et al., 2002; Farajollahi et al., 2005; Fechter-Leggett et al., 2012; Goddard et al., 2003; Micieli et al., 2013; Nelms et al., 2013a, 2013b; Phillips et al., 2006; Reisen et al., 2006; Unlu et al., 2010; Zuñiga et al., 2023). A minimal infection rate (MIR) described as number of infected individuals per 1000 individuals was determined in 13 articles (Anderson et al., 2006, 2008, 2012; Baqar et al., 1993; Calzolari et al., 2013; Dinu, 2015; Dohm et al., 2002; Farajollahi et al., 2005; Fechter-Leggett et al., 2012; Goddard et al., 2003; Micieli et al., 2013; Nelms, et al., 2013a; Nelms, 2013b). The average MIR found is 2.45/1000 individuals from all mosquitoes species combined. The MIR of *Culex pipiens* is not significantly different from the total mosquito MIR (*Culex pipiens* mean MIR of 1.37/1000 individuals, p-value = 0.26, two-sided T-test with equal variances). Lab and field observations were not significantly different (MIR: Lab: 3.02; Field: 1.52, p-value = 0.34, two-sided T-test with equal variances). Vertical transmission rate (VTR) was determined in seven articles (Anderson et al., 2008, 2012; Dinu, 2015; Fechter-Leggett et al., 2012; Nelms et al., 2013a, 2013b; Reisen et al., 2006). The VTR is calculated as the total number of WNV infected female mosquitoes that transmit WNV to their progeny divided by the total number of WNV infected females that lay eggs (Nelms et al., 2013a). The average VTR is 14.1% with no significant difference for *Culex pipiens* (VTR: 11.5%, p-value = 0.75). Lab and field VTRs were not significantly different (VTR: Lab: 4.0%, Field: 20.85%, p-value = 0.10, two-sided T-test with equal variances).

Direct avian transmission routes

Information about direct bird-to-bird transmission of WNV was found in 11 papers , see Supplementary files Tab S2. Transmission through direct contact between birds was reported in 9 papers(Escribano-Romero et al., 2009; Komar et al., 2003; Langevin et al., 2001; McClean et al., 2001; Nemeth et al., 2006a, 2006b, 2009; Reisen et al., 2006; Sotelo et al., 2011). Direct contact transmission between birds is defined as transmission of WNV through close contact between an infectious and susceptible bird individual . Transmission through direct contact was found in 4 of the 9 papers (Escribano-Romero et al., 2013; Komar et al., 2003; Langevin et al., 2001; McClean et al., 2001). Effective contact transmission was recorded in Ring-billed Gulls, Blue Jays, American Crows, Black-billed Magpies and Red-legged Partridges, see Table 5. The found R_0 are all above one when contact transmission was found.

Table 5. transmission rates and basic reproduction number of contact transmission experiments for different bird species

Species	Cage transmission rate	Basic Reproduction Number (R_0)
Ring-Billed Gull	1	5.45
Blue Jays	1	5.44
American Crow	0.86	4.66
Black-Billed Magpie	0.7	3.81
Red-Legged Partridge	1	5.44

Effective transmission of WNV through scavenging was found in 4 species (Great Horned Owl, American Crow, American Kestrel and Eastern Screech Owl) with a mean transmission rate of 0.546 (Komar et al., 2003; Nemeth et al., 2006a, 2006b) . The corresponding R_0 is 2.97. WNV persistence in carcasses was reported in 1 paper looking at American Crows and House Sparrows (Panella et al., 2005). Oral WNV persistence was determined between 83 and 90% day 1 pi in American Crows and between 70 and 90% in House Sparrows, whereas WNV prevalence was between 80 and 100% on day 4 pi in American Crows and 83% 3 days pi in House Sparrows. Cloacal WNV persistence was between 69 and 90% on day 1 pi in American Crows and between 56 and 67% in House Sparrows and changed to levels between 0 and 80% on day 4 pi in American crows and between 50 and 100% on day 2 pi in House Sparrows. Bird carcasses can persist in rural and urban areas with 25% of the bird carcasses still present 6 days after they died (Ward et al., 2006). WNV infection through an aqueous solution was 100% effective in American Crows and House Sparrows (Komar et al., 2003). Finally, direct evidence of infection through faeces was not found in any paper.

WNV persistence in birds

WNV persistence in birds was investigated in 26 papers, see Supplementary files Tab S3. The articles contained 40 different bird species. All papers performed experimental research (Busquets et al., 2012; Calzolari et al., 2013; Del Amo et al., 2014; Dridi et al., 2013; Hofmeister et al., 2015, 2016, 2017, 2018; Holicki et al., 2020; Jiménez de Oya et al., 2018; Komar et al., 2003; Kunkel et al., 2022; Lim et al., 2014, 2015; Llorente et al., 2023; Nemeth et al., 2006, 2011, 2017; Pérez-Ramírez et al., 2018; Reemtsma et al., 2022; Senne et al., 2000; Spedicato et al., 2016; Swayne et al., 2000; VanDalen et al., 2013; Wheeler et al., 2012; Ziegler et al., 2013), although one paper also used field evidence (Calzolari et al., 2013). WNV viremia were detected in 25 papers (Busquets et al., 2012; Calzolari et al., 2013; Del Amo et al., 2014; Hofmeister et al., 2015, 2016, 2017, 2018; Holicki et al., 2020; Jiménez de Oya et al., 2018; Komar et al., 2003; Lim et al., 2014, 2015; Llorente et al., 2023; Nemeth et al., 2006, 2011, 2017; Pérez-Ramírez et al., 2018; Reemtsma et al., 2022; Senne et al., 2000; Spedicato et al., 2016; Swayne et al., 2000; VanDalen et al., 2013; Wheeler et al., 2012; Ziegler et al., 2013). WNV viremia generally peaked around day three post-infection (pi) in the articles. Generally, WNV viremia was not detected after one week pi, although an average time cannot be determined (seven papers discussing this period stopped their research before WNV viremia levels became undetectable (Hofmeister et al., 2016, 2018; Komar et al., 2003; Lim et al., 2014; Nemeth et al., 2011; Reemtsma et al., 2022; Ziegler et al., 2013)). WNV RNA can also remain in the organs of birds after WNV viremias disappear. WNV RNA presence in bird organs was stated in 16 papers (Calzolari et al., 2013; Del Amo et al., 2014; Dridi et al., 2013; Hofmeister et al., 2016, 2017, 2018; Holicki et al., 2020; Jiménez de Oya et al., 2018; Kunkel et al., 2022; Lim et al., 2014, 2015; Llorente et al., 2023; Pérez-Ramírez et al., 2018; Reemtsma et al., 2022; Spedicato et al., 2016; Wheeler et al., 2012). WNV RNA was mostly found in the spleen (10 times), the kidneys (8 times), the brains (8 times), the heart (6 times) and the liver (5 times). It is difficult to determine how long WNV RNA remains in the organs. Still, WNV RNA was also detected 36 weeks pi in House Sparrows (*Passer domesticus),* indicating that long-term WNV persistence is possible.

WNV reintroduction by migratory birds

14 papers were selected to give insight in the WNV prevalence of migratory birds, see Supplementary files Tab S4. At least 133 species were investigated in these 14 papers (Allison et al., 2004; Ayadi et al., 2019; Dusek et al., n.d., 2009; Giglia et al., 2022; Hull et al., 2006; Jourdain et al., 2008; Lim et al., 2018; Llopis et al., 2015; Lopez et al., 2008; Mancuso et al., 2022; Mencattelli et al., 2022; Reisen et al., 2010, 2013). 2 papers researched WNV viremia prevalence in migratory birds upon return or at a stopover (Ayadi et al., 2019; Dusek et al., 2009). Both papers did not find any infected birds. Overwintering resident birds were investigated in one paper where also no infected birds were found (Dusek et al., 2010). WNV RNA persistence in bird organs from migratory birds or overwintering residents birds in spring was reported in five papers (Giglia et al., 2019; Mancura et al., 2022; Mencattelli, 2022; Reisen et al., 2010, 2013). Big differences in WNV RNA persistence was found ranging from 0.99 to over 60%. A mean RNA persistence of WNV in returning migratory birds and overwintering resident birds can not be made with these findings. Finally, WNV antibody prevalence was investigated by six papers which results in an average WNV antibody prevalence in returning migratory birds of 10.6% (Ayadi et al., 2019; Dusek et al., 2009; Jourdain et al, 2008; Llopis et al., 2015; López et al., 2008; Reisen et al., 2010). Antibody presence is an indication that WNV RNA could also be present in bird organs. Seven papers reported WNV antibody prevalence in overwintering resident birds in spring with an average of 27.48% (Allison et al., 2004; Dusek et al., 2010; Hull et al., 2006; Jourdain et al., 2008; Lim et al., 2018; Llopis et al., 2015; López et al., 2008). The WNV antibody prevalence in both groups is not significantly different (P-value = 0.148, two-sided T-test with equal variances).

Model-based analyses

General model output

The R_0 remained equal to zero before May with cold and average temperatures in April, see Figure 2. When April temperatures were relatively high, R_0 was already above 0 in April enabling WNV transmission early on in he transmission season of WNV. Across all temperature scenarios, R_0 increased above 1 in May and reached maximum R_0 in August with values above 10.

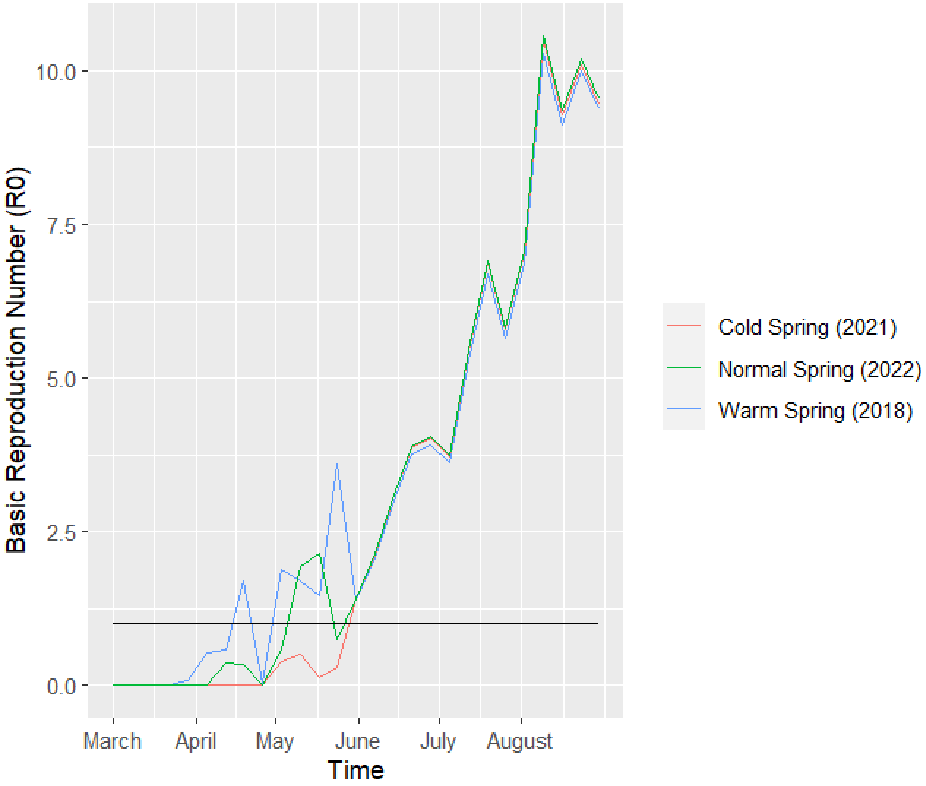

Figure 2. Basic reproduction number (R_0) over time. Different colours indicate different years with different temperatures in April (red: cold spring (2021), green: normal spring (2022), green: warm spring (2018))

Population dynamics are shown in Figure 3. Mosquito population dynamics in the different years were similar. Mosquito populations remain stable till June before it exponentially grows till the end of August. Bird population reached a maximum population size at the start of August when the breeding season came to an end. Afterwards, the bird population decreased because the breeding season was over.

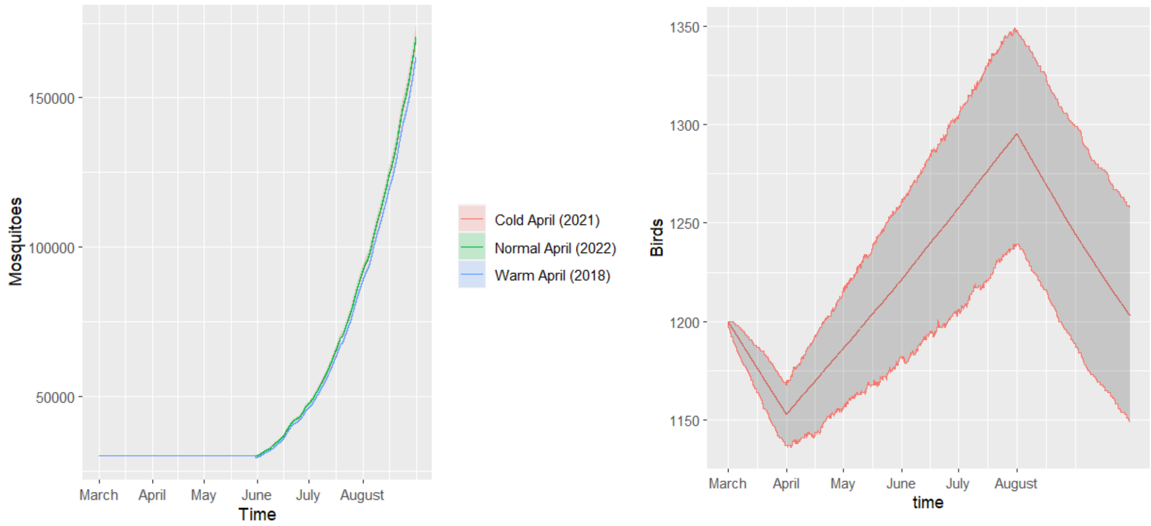

Figure 3. Population dynamics of mosquitoes (left) and birds (right). Lines indicate mean and shadows indicate standard deviation. Different colours indicate different years with different temperatures in April (red: cold April (2021), green: normal April (2022), blue: warm April (2018)

Initial mosquito and bird prevalence of WNV

The probability that infected mosquitoes or birds were still present in the population at the start of the transmission season is shown in Figure 4. Low initial WNV prevalence in the mosquito population already resulted in a high probability that infected mosquitoes will be present at the start of the transmission season. At an initial WNV mosquito prevalence of 0.02% the fraction of infected mosquitoes left was equal to 0.992 with no initial WNV prevalence in the bird population. With only 0.0033% initial WNV prevalence in the mosquito population and no initial bird prevalence the fraction is 0.518. The fraction of infected birds is lower with a maximum om 0.19 when initial mosquito prevalence is 0.020% and initial bird prevalence 10%. An increase in initial bird prevalence does not influence the probability that infected mosquitoes are still present at the start of the transmission season. However, initial bird prevalence appears to have an influence on the probability that infected birds are present at the start of the transmission season. This relation is positive, but a clear pattern cannot be confirmed (Figure 4).

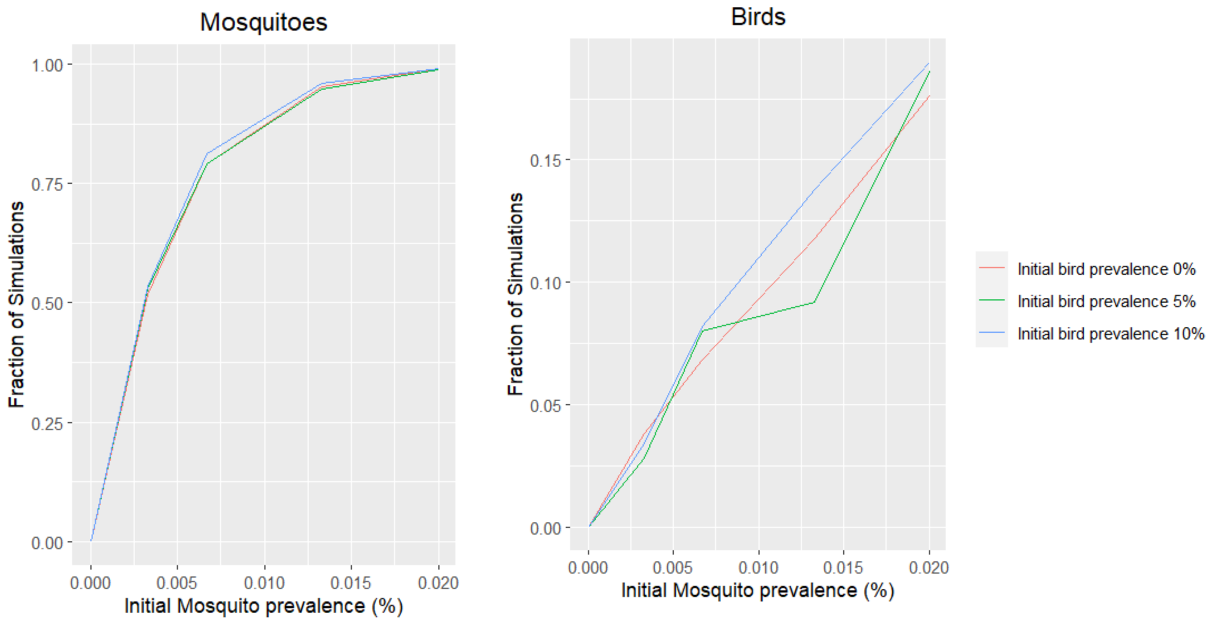

Figure 4. Fraction of simulations that have infected mosquitoes (left) or birds (right) left at the start of the transmission season plotted against the initial prevalence of WNV in the mosquito population. Colours indicate different initial prevalences of WNV in the bird population (red: 0%; green: 5%; blue: 10%)

Final sizes (the total number of infected individuals divided by the total number of individuals that lived in the population during the simulation period) of the mosquito and bird population for WNV at the end of the transmission season are presented in Figure 5. The WNV final size in the mosquito population is increasing with initial mosquito prevalence from 0 to 0.014. Higher initial bird prevalence result in lower final sizes in the mosquito population. The final size in birds increased with both higher initial mosquito and bird prevalences, but final sizes are evidently higher. The highest final size in the bird population was 0.87-0.88 at an initial mosquito prevalence of 0.02%. A maximum final size in the birds population was not reached yet, but almost all birds left in the population will have been infected.

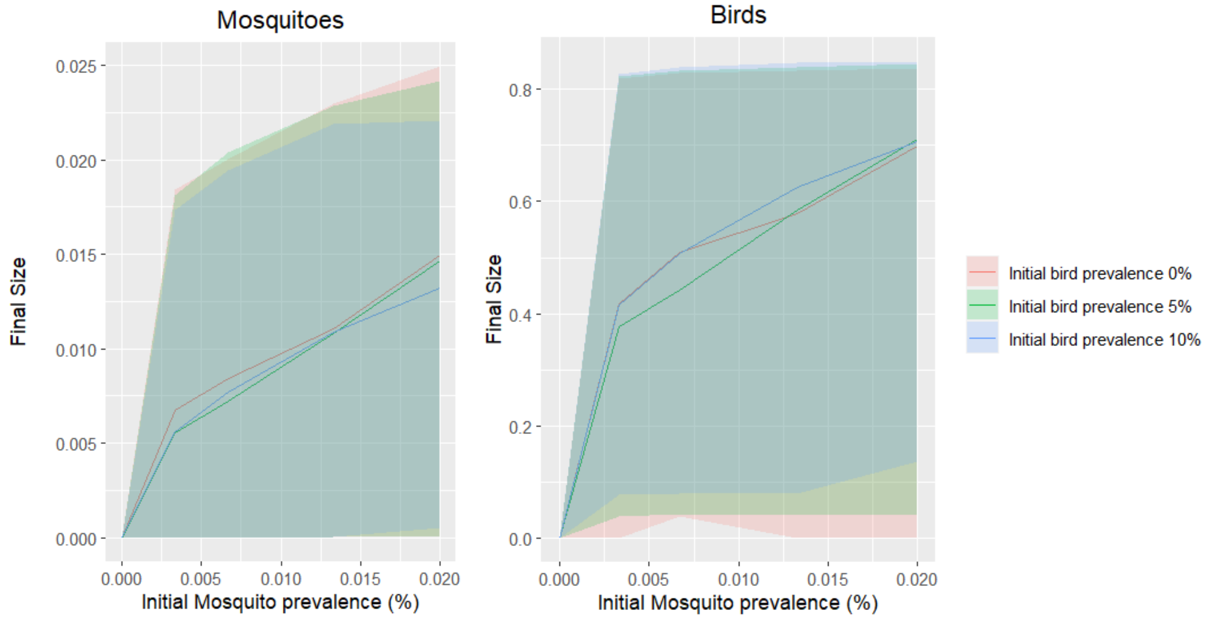

Figure 5. WNV Final sizes of the mosquito population (left) and bird population (right) plotted against the initial mosquito prevalence. Lines indicate mean values and shadows indicate the 95% confidence interval. Colours indicate different WNV initial bird prevalences (red: 0%; green: 5%; blue: 10%)

Temperatures in April

The probability of a WNV outbreak was bigger when temperatures are higher during April, see figure 6. Especially the probability that there is an infected bird at the start of the transmission season was dependent on the temperatures in April. The probability that an infected mosquito was present in the population is independent on the temperatures in April.

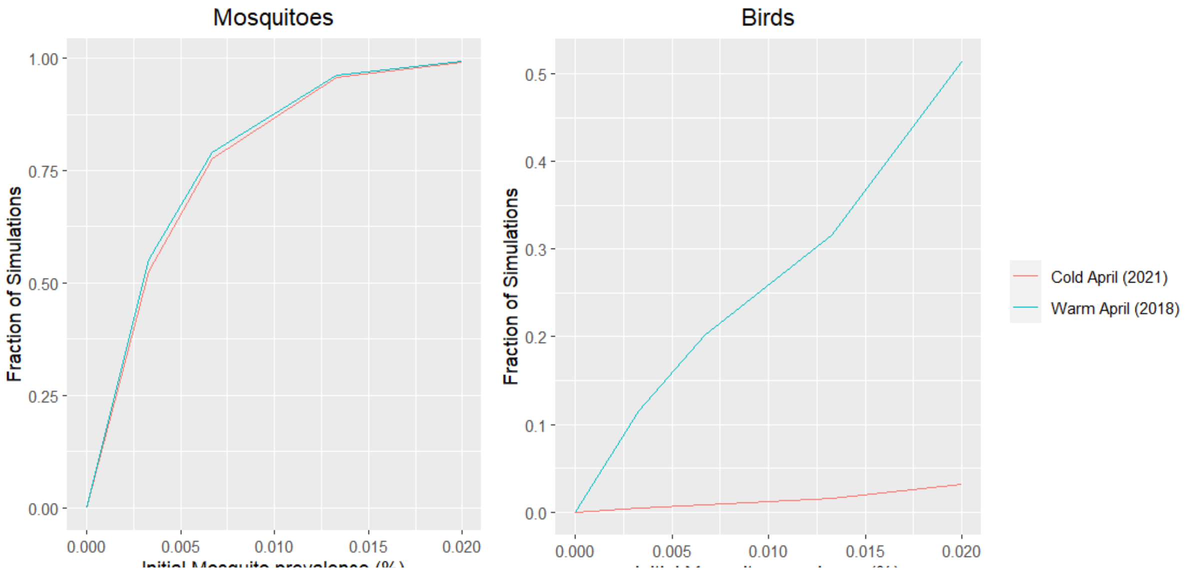

Figure 6. Fraction of simulations that have infected mosquitoes (left) or birds (right) left at the start of the transmission season plotted against the initial prevalence of WNV in the mosquito population. Colours indicate different initial prevalences of WNV in the bird population (red: Cold April (2021); blue: Warm April (2018))

The final size of the outbreak also differs with differences in April temperatures, see figure 7. Final sizes tend to be higher with higher temperatures in April.

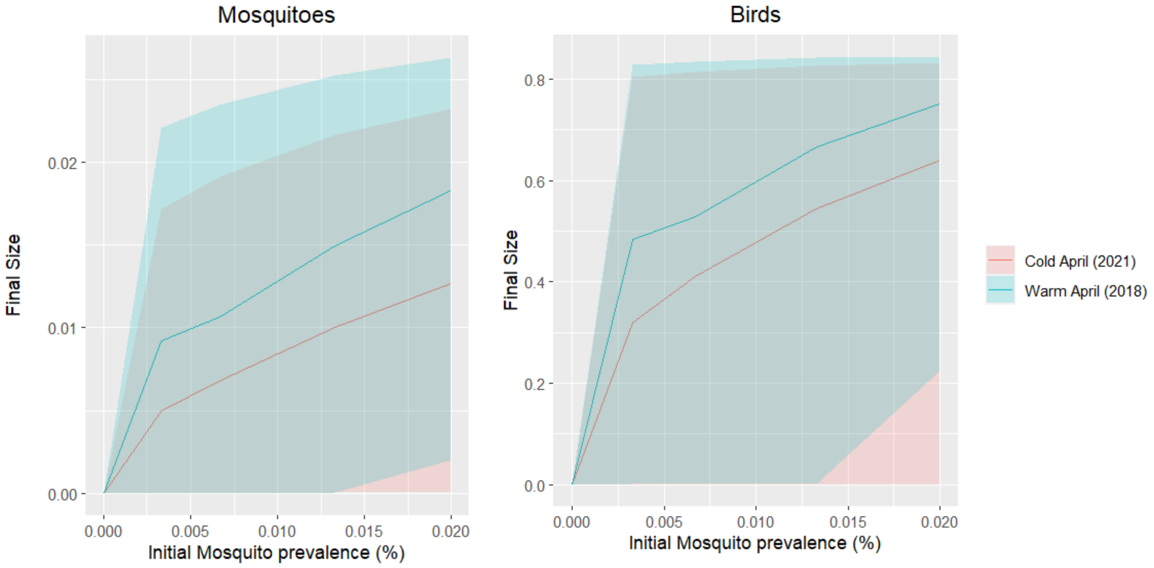

Figure 7. WNV Final sizes of the mosquito population (left) and bird population (right) plotted against the initial mosquito prevalence. Lines indicate mean values and shadows indicate the 95% confidence interval. Colours indicate different temperatures in April (red: Cold April (2021); blue: Warm April (2018))

Start of the mosquito season

Earlier starts of the mosquito season did not influence the probability that infectious mosquitoes were present at the start of the WNV transmission season, see Figure 8. The fraction of infected birds at the start of the transmission season was also not different with changes in the start of the mosquito season for initial mosquito prevalences below 0.015%. At initial mosquito prevalences for WNV above 0.015% an earlier start of the mosquito season resulted in higher probability that there was at least one bird infected at the start of the transmission season.

The start of the mosquito season did not have a significant influence on the final size in both the mosquito and bird population, see Figure 9. However, a trend was visible that with an early start of the mosquito season final sizes in both the mosquito and bird population was higher.

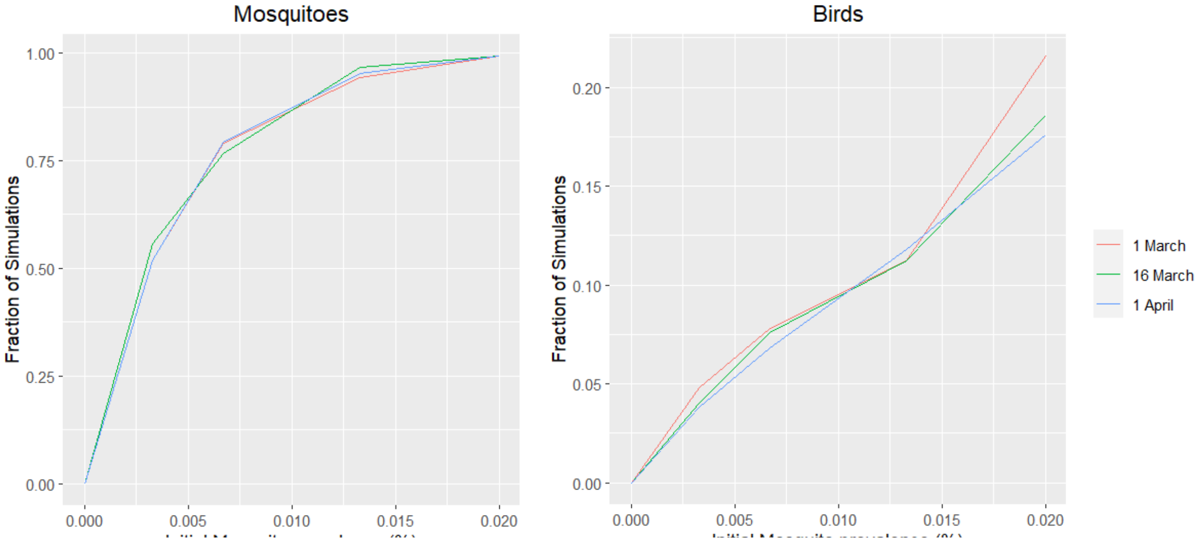

Figure 8. Fraction of simulations that have infected mosquitoes (left) or birds (right) left at the start of the transmission season plotted against the initial prevalence of WNV in the mosquito population. Colours indicate different starts of the mosquito season (red: 0%; green: 5%; blue: 10%)

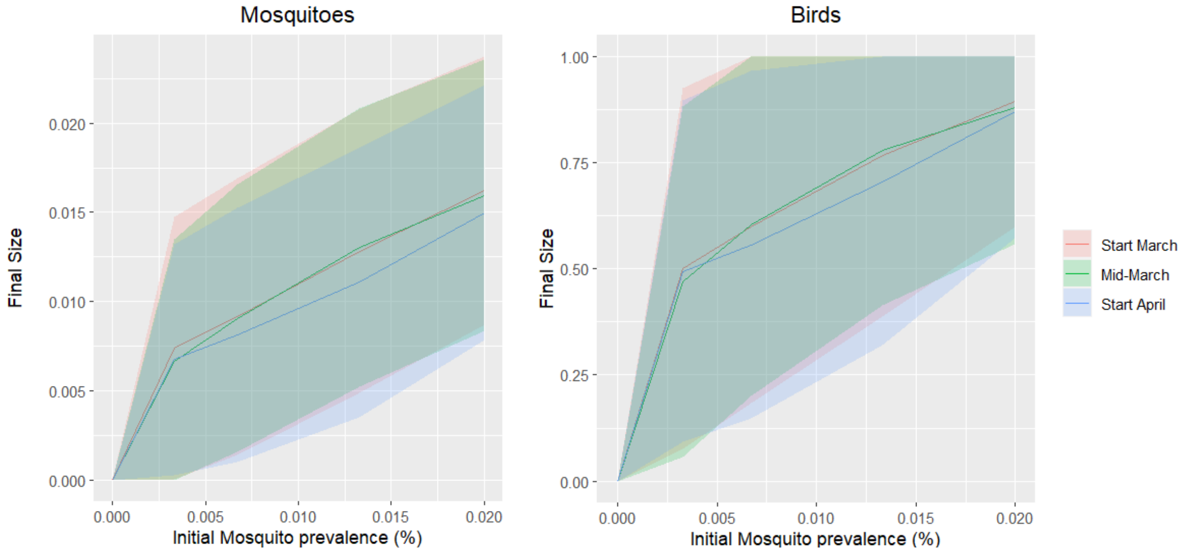

Figure 9. WNV Final sizes of the mosquito population (left) and bird population (right) plotted against the initial mosquito prevalence. Lines indicate mean values and shadows indicate the 95% confidence interval from the mean. Colours indicate different WNV initial bird prevalences (red: 0%; green: 5%; blue: 10%)

Discussion

This research investigated the efficiency of different WNV overwintering mechanisms and the risk of an outbreak of WNV during the summer. Efficiency of the overwintering mechanisms was not clearly found for all overwintering mechanisms. Vertical transmission of WNV in mosquitoes was found with low efficiency, while WNV RNA was found for up to 36 weeks pi in birds, although it is unclear if this WNV RNA can still be infectious. Limited evidence is available for direct avian transmission, but the knowledge present indicates efficient WNV transmission in birds through scavenging or direct contact with basic reproduction numbers that are above one Knowledge about WNV persistence in returning migratory birds and overwintering resident birds is still limited. Model simulations showed that low WNV prevalence in the mosquito population at the start of the mosquito season can already result in a high probability of a WNV outbreak during the summer with almost all birds infected in August. Spring temperatures influence the probability of an outbreak and final size in mosquitoes with higher temperatures resulting in bigger probabilities and final sizes. Differences in initial WNV prevalence in the bird population and earlier starts of the transmission did not have an impact on the probability and size of the outbreak. A limitation of this research was that temperature-dependent parameters were extrapolated for temperatures below 15 °C. Since the temperature plays a crucial role in early spring it would be useful if more research will be performed on the mortality, birth and activity of mosquitoes at lower temperatures to get a better insight how WNV can spread and how mosquito dynamics change.

The high probability of a WNV outbreak with low initial mosquito prevalences was due to long mosquito lifespans at low temperatures (Shocket et al., 2020). At low temperatures the adult lifespan of *Culex pipiens* was estimated to be longer with a mean lifespan of 97 days at temperatures below 15 ˚C (Shocket et al., 2020; Ruybal et al., 2016, Andreadis et al., 2014; Ciotta et al., 2014). Other papers found comparable mortality rates for *Culex pipiens* (Moser et al., 2023; Rubel et al., 2008) at low temperatures. These estimates are based on laboratory experiments. Mosquito mortality rates may be different in nature, especially because there is no predation in laboratories and environmental conditions are more stable in experimental situations. A field experiment regarding *Culex pipiens* lifespan showed comparable lifespans in adult mosquitoes during the fall when temperatures are more comparable with spring temperatures (Papadopoulos et al., 2016). Shorter lifespans around 10 days were found in another field experiment (Lambert et al., 2022). However, this data consists of other mosquito species and the catching period is unclear making it hard to compare the results to the laboratory experiments with respect to the effect of temperature. Knowledge so far is in line with the option that mosquitoes can survive till the transmission season of WNV, meaning that low mosquito prevalences at the start of the mosquito season are already enough to result in an outbreak in summer.

The simulations in the model only last for one transmission season and it is assumed that there are no WNV immune birds in the population at the start of the simulation. Since the bird population is fully susceptible and R_0 has high values above 1 for the period May – August almost the entire population is infected at the end of the transmission season. However, this does not mean that the same WNV spread will be seen the next year, even with comparable temperatures. Returning migratory birds can be infected at their overwintering location. This means that the birds can be resistant for WNV. The fraction of returning birds that are WNV resistant is not clearly found yet. Limited evidence is available on WNV persistence in returning migratory birds and overwintering resident birds. Although it is known that WNV RNA can remain in avian organs for a longer period of time (Wheeler et al., 2012; Calzolari et al., 2013), there is no evidence yet that showed if this WNV RNA This information can be useful to better understand overwintering mechanisms using direct avian transmission, but can also give better insight how WNV spreads during summer and how likely overwintering occurs through either migratory or resident birds.

To conclude, the probability of a WNV outbreak during the summer is already high with low initial prevalences in the mosquito population. These outbreaks result in high level of infection in the bird population. Initial bird prevalences for WNV did not have an impact on the probability and size of an outbreak. This means that for WNV overwintering the initial prevalence in the mosquito population seems to be of greater importance than in the bird population. Therefore, it is useful that further research should more focus on mosquito prevalences than on bird prevalences at the start of spring. Especially more knowledge on the mosquito mortality at lower temperatures could help to understand how WNV can persist when transmission is halted. This can also be useful for other mosquito-borne infectious diseases.

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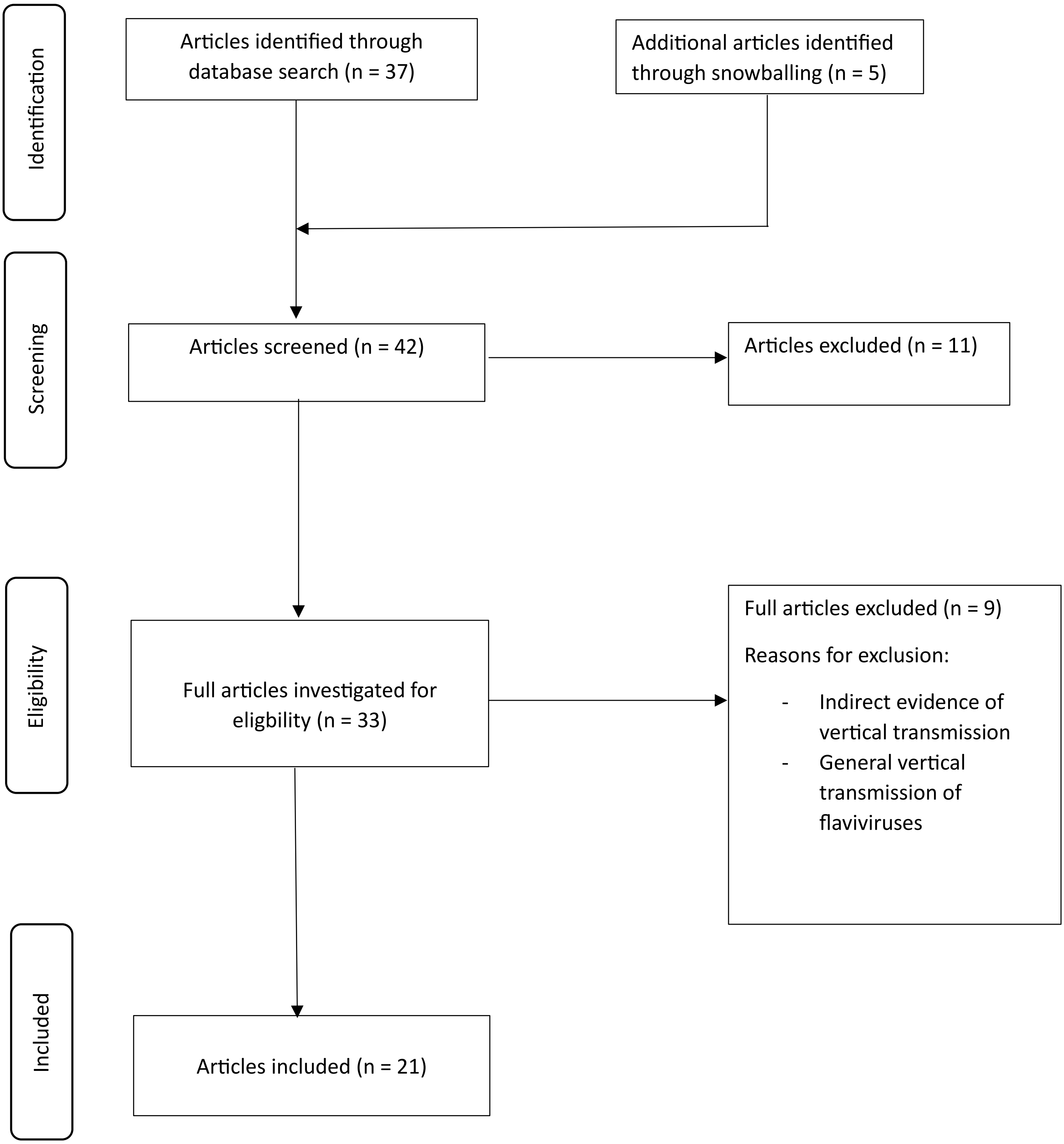
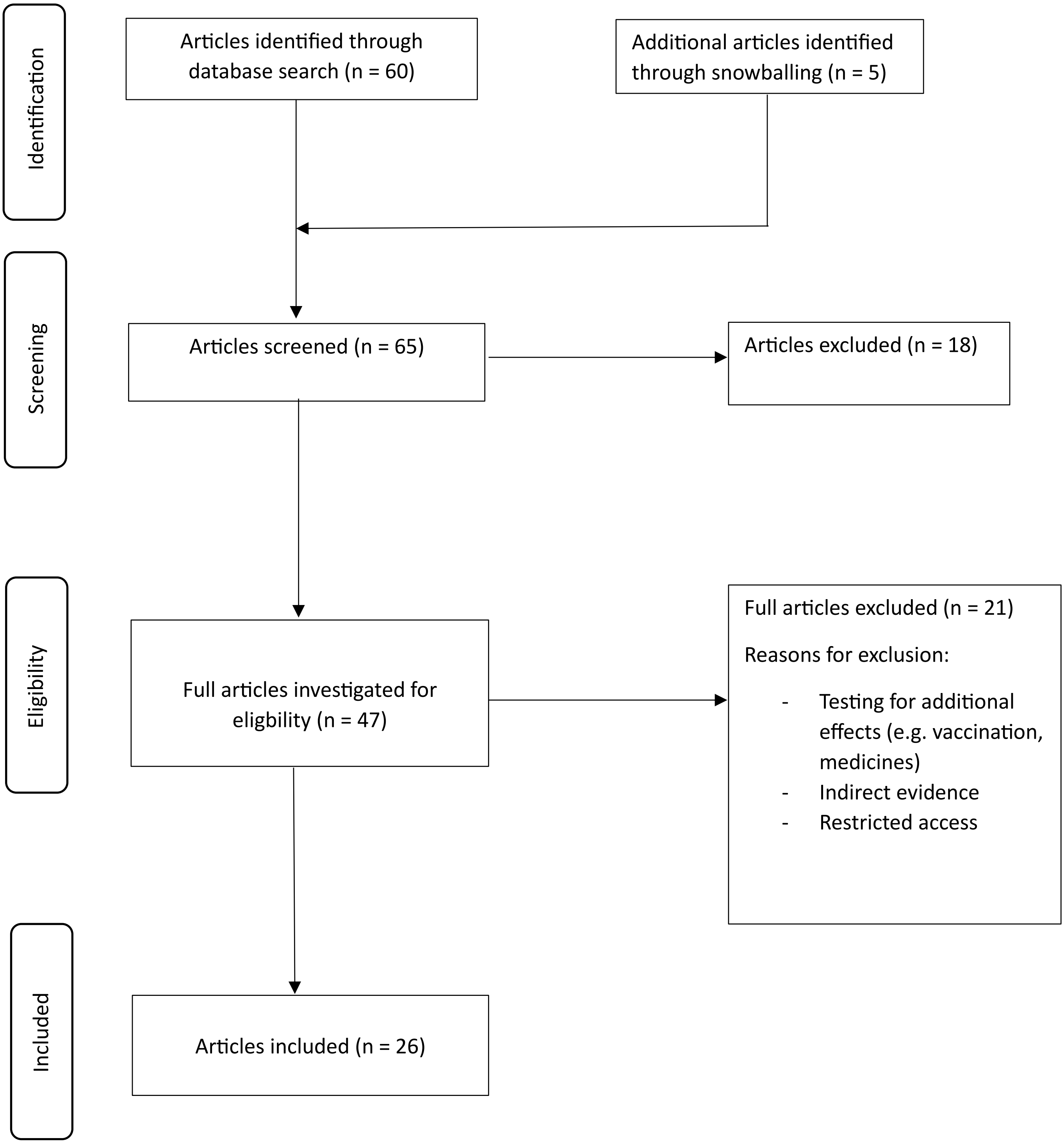
Appendix

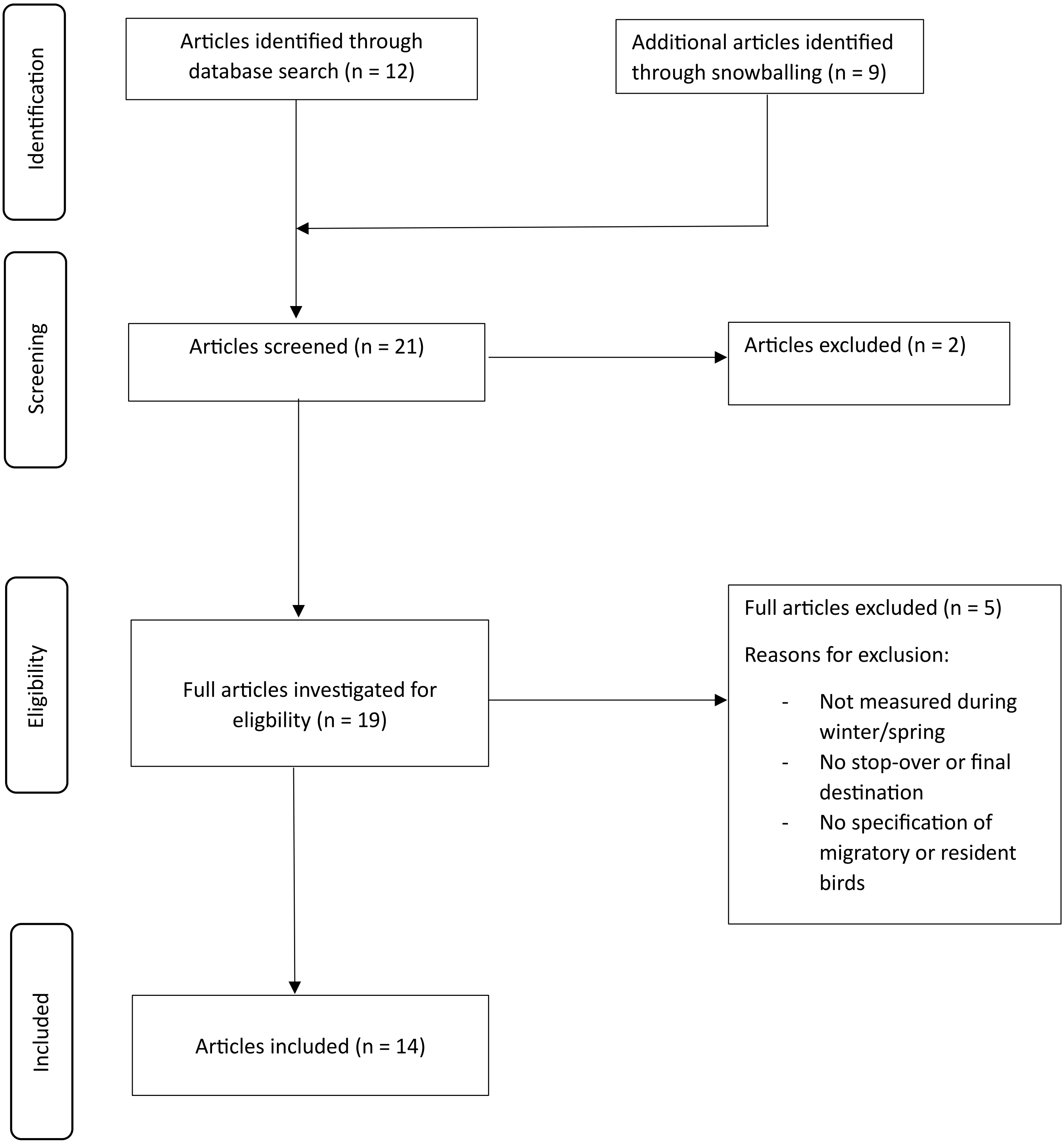
S1: PRISMA flow chart for WNV vertical transmission in mosquitoes



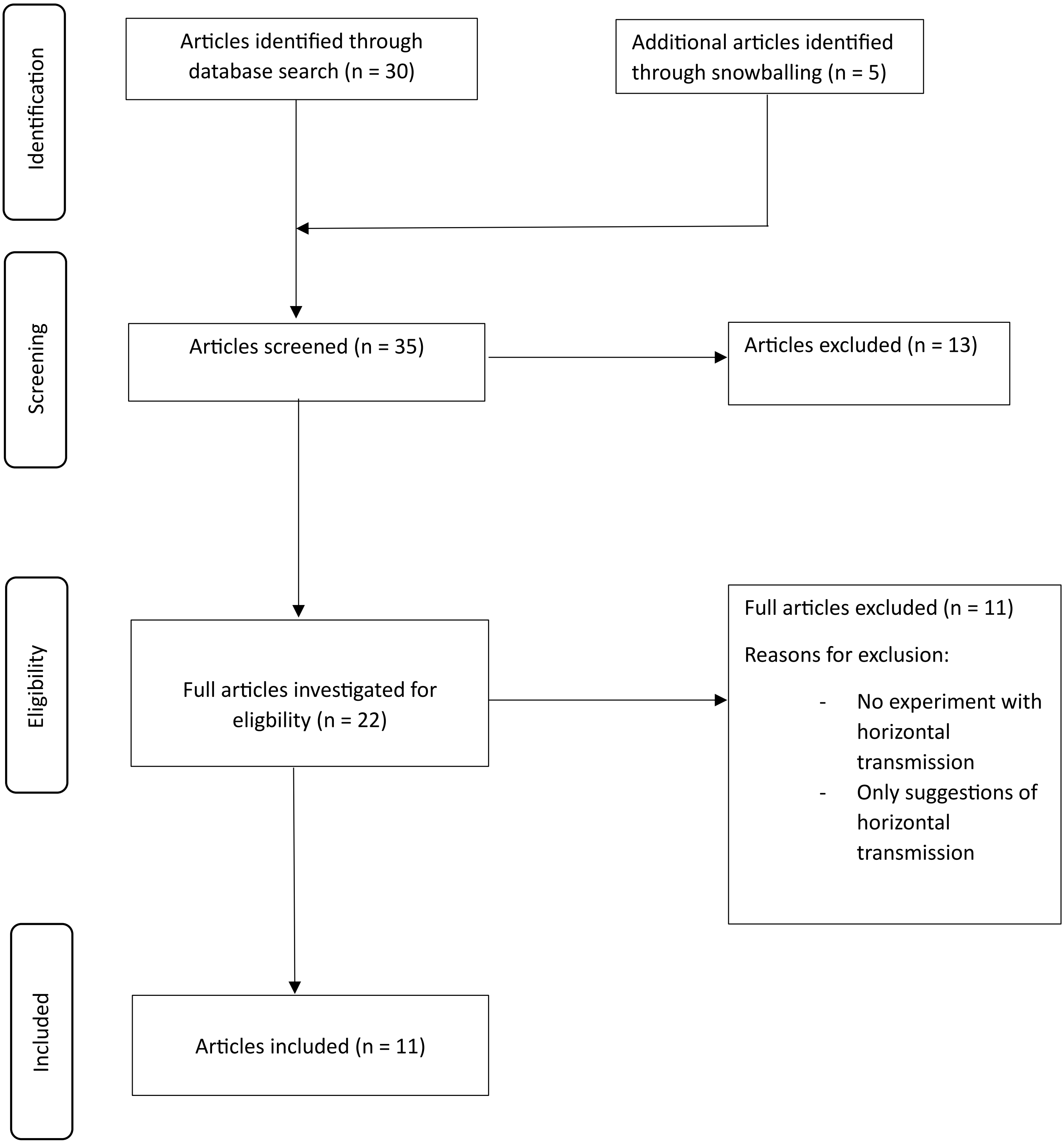
S2: Prisma flow chart for WNV persistence in birds



S3: PRISMA flow chart for WNV reintroduction by migratory birds



S4: PRISMA flow chart for direct avian transmission of WNV



S5: Table with model parameters and their source

Parameters	**Symbol**	**Function**	**Source**
Larval emerge rate (day^{-1})	$\alpha_L(T)$	f(T) = $1.70 * 10^{-4} * (T - 9.4) * \sqrt{39.6 - T}$	Shocket et al., 2020; Li et al., 2017; Ruybal et al., 2016; Madder et al., 1983; Tekle et al., 1960
Larval-to-Adult survival probability	pLA(T)	f(T) = $3.60 * 10^{-3} * (T - 7.8) * (T - 38.4)$	Shocket et al, 2020; Ruybal et al., 2016; Ciota et al., 2014; Loetti et al., 2011; Mpho et al., 2002a; Mpho et al., 2002b; Madder et al, 1983; Tekle et al., 1960
Larval time (day)	t(T)	f(T) = $\frac{1}{3.76*10^{-5}*T*(T-7.8)*\sqrt{38.5-T}}$	Shocket et al, 2020; Ruybal et al., 2016; Ciota et al., 2014; Loetti et al., 2011; Mpho et al., 2002a; Mpho et al., 2002b; Madder et al, 1983; Tekle et al., 1960
Larval mortality rate (day^{-1})	$\mu_L(T)$	f(T) = $\frac{Ln(1-pLA(T))}{-t(T)}$	-
Adult emerge rate (day^{-1})	$\alpha_A(T)$	f(T) = $3.76 * 10^{-5} * T * (T - 7.8) * \sqrt{38.5 - T}$	Shocket et al, 2020; Ruybal et al., 2016; Ciota et al., 2014; Loetti et al., 2011; Mpho et al., 2002a; Mpho et al., 2002b; Madder et al, 1983; Tekle et al., 1960
Adult mortality rate (day^{-1})	$\mu_A(T)$	for T > 15: f(T) = $\frac{1}{-4.86*T+169.8}$ for T ≤ 15: f(T) = 0.0103	Shocket et al., 2020; Ruybal et al., 2016, Andreadis et al., 2014; Ciotta et al., 2014
Biting rate (day^{-1})	k(T)	f(T) = $1.70 * 10^{-4} * (T - 9.4) * \sqrt{39.6 - T}$	Shocket et al., 2020; Li et al., 2017; Ruybal et al., 2016; Madder et al., 1983; Tekle et al., 1960
Transmission probability mosquito-to-bird	p_{MB}	1	Wonham et al., 2004
Transmission probability bird-to-mosquito	p_{BM}	0.125	Wonham et al., 2004
Mosquito transition rate from infected to infectious (day^{-1})	$\sigma_M(T)$	f(T) = $7.38 * 10^{-5} * T * (T - 11.4) * \sqrt{45.2 - T}$	Shocket et al., 2020; Kilpatrick et al., 2008; Dohm et al. ,2002
Bird birth rate (day^{-1})	α_B	0.0023	Sovon, 2023
Bird death rate (day^{-1})	μ_B	0.0013	Sovon, 2023
Bird transition rate from infected to infectious (day^{-1})	σ_B	0.667	Rubel et al., 2008
Bird recovery rate (day^{-1})	γ_B	0.1274	Rubel et al., 2008; Weisenböck et al., 2002
WNV death rate for birds (day^{-1})	λ_B	0.0546	Rubel et al., 2008; Weisenböck et al., 2002

S6: Adult mosquito birth rates for the years 2018-2022

	Year		
Day	2018	2021	2022
1-mrt	0	0	0
2-mrt	0.0003097	0	0
3-mrt	0.0001711	0	0
4-mrt	0.0004156	0	0
5-mrt	0.0012812	0	0
6-mrt	0.0009254	0	0
7-mrt	0.0006485	0	0
8-mrt	0.0007219	0	0
9-mrt	0.0011835	0	0
10-mrt	0.0036501	0	0
11-mrt	0.0032778	0	0
12-mrt	0.0031298	0	0
13-mrt	0.0010407	0	0.000129431
14-mrt	0.0010378	0	0.000331423
15-mrt	0.0013681	0	0.000236524
16-mrt	0.0003929	0	0.000367329
17-mrt	7.84E-05	0	0.000243368
18-mrt	5.07E-05	0	0.000237569
19-mrt	0	0	0.000271555
20-mrt	0.000423	0	9.40E-05
21-mrt	0.0003668	0	0.000281485
22-mrt	0.0013853	0	0.000471661
23-mrt	0.0015252	0	0.000606442
24-mrt	0.0018654	0	0.000566746
25-mrt	0.0013878	0	0.00049587
26-mrt	0.0009675	0	0.000624424
27-mrt	0.0014561	0	0.000553692
28-mrt	0.0014721	0	0.000422115
29-mrt	0.0015928	0	0.000415694
30-mrt	0.002861	0.00063724	0.000239594
31-mrt	0.0022716	0.00182913	1.46E-06
1-apr	0.0009928	0.00165291	5.93E-06
2-apr	0.0023595	0.0006736	2.33E-05
3-apr	0.0059222	0.00093676	4.63E-05
4-apr	0.0045858	0.00074326	0.00030297
5-apr	0.0017058	0.0002431	0.000607416
6-apr	0.003442	5.16E-05	0.000628205
7-apr	0.0075837	0.00020152	0.000490965
8-apr	0.0088191	0.00055491	0.000253277
9-apr	0.0073469	0.00109905	0.000190932
10-apr	0.0112051	0.00055032	0.00023709
11-apr	0.008392	0.00032185	0.000556464
12-apr	0.0075102	0.00020087	0.001184637
13-apr	0.0076659	0.00040928	0.002479047

14-apr	0.0076447	0.00036254	0.002966832
15-apr	0.0102943	0.00051218	0.002104121
16-apr	0.0092617	0.00085062	0.002697114
17-apr	0.0118739	0.00092647	0.003274489
18-apr	0.0187888	0.0012989	0.004147228
19-apr	0.0278267	0.00159976	0.004601404
20-apr	0.0263894	0.00203203	0.004697022
21-apr	0.021067	0.00131291	0.005627809
22-apr	0.0281653	0.00087505	0.00658618
23-apr	0.0178829	0.00100445	0.008152987
24-apr	0.0168395	0.00114586	0.007128599
25-apr	0.014491	0.00090143	0.004525558
26-apr	0.0115123	0.00092057	0.004667139
27-apr	0.0127517	0.0015155	0.003972035
28-apr	0.0149078	0.00213714	0.004605635
29-apr	0.011695	0.00127749	0.004097103
30-apr	0.0101873	0.00137196	0.003789861
1-mei	0.0076062	0.00122753	0.004368397
2-mei	0.0133436	0.00100662	0.005745127
3-mei	0.0105023	0.00190638	0.005010273
4-mei	0.0127698	0.00161313	0.005335962
5-mei	0.0194252	0.00103956	0.007540687
6-mei	0.0279483	0.00089069	0.009706914
7-mei	0.0328243	0.00078102	0.011116065
8-mei	0.0380734	0.00212139	0.008522464
9-mei	0.0348497	0.00593682	0.013426394
10-mei	0.0191294	0.00942242	0.01920282
11-mei	0.0208699	0.00916086	0.019919237
12-mei	0.034611	0.00869162	0.01713893
13-mei	0.0253148	0.00864755	0.017881162
14-mei	0.0348809	0.00701123	0.020400829
15-mei	0.0376646	0.00723656	0.027730249
16-mei	0.0265384	0.00812141	0.030131454
17-mei	0.0162784	0.00814941	0.033688108
18-mei	0.0149262	0.00834541	0.038073521
19-mei	0.0160789	0.00753044	0.034291265
20-mei	0.0283497	0.00962762	0.023798236
21-mei	0.0378136	0.01085192	0.023944949
22-mei	0.0365711	0.00808589	0.029443659
23-mei	0.0391038	0.00953123	0.032505334
24-mei	0.0406068	0.00982328	0.022356671
25-mei	0.0440882	0.00793815	0.024622374
26-mei	0.0513336	0.00882314	0.03032516
27-mei	0.0490547	0.00851943	0.023937803
28-mei	0.0546296	0.01058131	0.018949976
29-mei	0.0532966	0.01175801	0.014642315
30-mei	0.0512653	0.01454975	0.015227909
31-mei	0.0484803	0.01935038	0.019568736

1-jun	0.0391967	0.02698466	0.018461069
2-jun	0.0362725	0.03352721	0.021746037
3-jun	0.0417695	0.03612302	0.031001333
4-jun	0.0380821	0.03641236	0.030454436
5-jun	0.03341	0.02746625	0.03161885
6-jun	0.0447125	0.02797016	0.024972655
7-jun	0.0501197	0.03099186	0.026326299
8-jun	0.0419518	0.0361519	0.028842194
9-jun	0.0452946	0.0397824	0.030475185
10-jun	0.0403632	0.04121557	0.035542756
11-jun	0.0371093	0.04077355	0.036146278
12-jun	0.0296145	0.03362889	0.031971477
13-jun	0.0287071	0.03365729	0.027069123
14-jun	0.0325408	0.04514655	0.028741063
15-jun	0.0387025	0.03860313	0.037457473
16-jun	0.0375806	0.05252678	0.038483363
17-jun	0.0330034	0.0603899	0.052345838
18-jun	0.035338	0.0560625	0.048585963
19-jun	0.0397416	0.04410918	0.029086302
20-jun	0.0439955	0.04348078	0.029908387
21-jun	0.0274244	0.02775298	0.03120448
22-jun	0.0233132	0.02847119	0.039762802
23-jun	0.0278071	0.02819456	0.054940584
24-jun	0.0273181	0.02943306	0.045807816
25-jun	0.0321949	0.03228865	0.044037081
26-jun	0.0316823	0.04312898	0.040790057
27-jun	0.03777	0.04599788	0.035644138
28-jun	0.0472385	0.04616657	0.040425923
29-jun	0.0422448	0.03752368	0.050753558
30-jun	0.0489664	0.02779941	0.042881584
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2-jul	0.0468722	0.03532476	0.041423082
3-jul	0.0440533	0.0423564	0.03804441
4-jul	0.0432527	0.04043855	0.037043973
5-jul	0.0409832	0.03733265	0.033782137
6-jul	0.0436301	0.04030074	0.035978511
7-jul	0.0408937	0.03914563	0.034407367
8-jul	0.0409541	0.04028272	0.038819318
9-jul	0.0378484	0.0387753	0.037315097
10-jul	0.0348676	0.03920343	0.035667732
11-jul	0.0397854	0.0420214	0.038766162
12-jul	0.0421295	0.04638978	0.05022489
13-jul	0.0403612	0.04358223	0.05052052
14-jul	0.0417387	0.0433837	0.036892202
15-jul	0.0492618	0.04068216	0.03678827
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1-aug	0.0532895	0.03361212	0.042130064
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7-aug	0.0683933	0.03797143	0.037056501
8-aug	0.052782	0.03624318	0.040344376
9-aug	0.0438943	0.03554893	0.045125623
10-aug	0.0383649	0.03615917	0.052746123
11-aug	0.0351621	0.03899752	0.0586353
12-aug	0.0487617	0.04310772	0.061587778
13-aug	0.044255	0.04019981	0.063002733
14-aug	0.0449083	0.04060121	0.065679417
15-aug	0.046437	0.04512311	0.059387928
16-aug	0.0440353	0.03294854	0.057839981
17-aug	0.0396976	0.03072776	0.048522716
18-aug	0.0407751	0.03689426	0.048866869
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22-aug	0.0477678	0.0399404	0.047840941
23-aug	0.0432215	0.04029159	0.054193968
24-aug	0.0324463	0.03612083	0.059123742
25-aug	0.0237273	0.0336376	0.063419591
26-aug	0.0284304	0.03419033	0.04725191
27-aug	0.0361799	0.03279531	0.040445681
28-aug	0.0367103	0.03576538	0.037857785
29-aug	0.0337202	0.03451861	0.039397874
30-aug	0.0310138	0.03834969	0.043670949
31-aug	0.025256	0.03524795	0.04215926