New approaches in habitat modelling of invasive mosquitoes for assessing the risk of pathogen transmissions and applications to *Aedes japonicus japonicus* and the West Nile virus in Germany

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by ANTJE KERKOW

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Summary

The international goods trade and passenger transport has caused a progressively severe problem with exotic mosquitoes reaching Europe and becoming invasive in many areas. These exotic mosquitoes may have the ability to transmit pathogens for a number of diseases affecting humans and other mammals, for which efficient vectors do not yet exist in the invaded area. Moreover, they can introduce the pathogens and trigger epidemics and epizootics that way. Climate change contributes to the fact that species adapted to tropical, subtropical and Mediterranean climates can increasingly establish themselves in Central Europe, while also dramatically increasing the risk of arbovirus-related disease outbreaks. The increased risk of infection is related in part to the virus incubation period, which is exponentially negatively correlated with the ambient temperature.

Due to the increased threat posed by the exotic mosquitoes, it is of utmost importance to map their expansion and to investigate their habitat preferences, dispersal behavior, population trends and vector potential. For this purpose, the monitoring project "CuliMo" and the research project "CuliFo" were founded, involving researchers from several scientific institutions in Germany. As a result of monitoring work and the citizen-based science project "Mückenatlas", a large amount of mosquito species data are now available in a database. My goal was to develop modelling methods that make optimal use of the species occurrence data from this joint project database, as well as biological and ecological knowledge about the invasive species, in order to predict their current and future potential distribution in Germany.

The models were set up for the Asian bush mosquito (*Aedes japonicus japonicus*), a species that was first detected in Germany in 2008, and has since spread rapidly in many parts of the country from the multiple locations where it was introduced. *Aedes j. japonicus* is a potential vector species for a wide range of pathogens harmful to humans and animals. Some laboratory experiments have demonstrated high transmission efficiency for West Nile virus (WNV), which has been detected in mosquito populations in Germany since 2018, and has resulted in incidences of disease in birds, horses and humans. This dissertation describes a stepwise process of modelling the current and future ecological niche of the Asian bush mosquito in Germany as well as the

generation of a flight model based on the created detailed habitat map, which is coupled to a WNV transmission model.

Chapter 2 presents the modelling of the climatic niche. For this purpose, a method was developed that can be applied even if only a small amount of occurrence data of the target species is available in the invaded area. Additional occurrence data of three native species, as well as temperature and precipitation data, were used to separate the climatic niche of the invasive species from the niches of the native species by machine learning. The model was trained with a support vector machine and mosquito data from 2011 to 2014, and evaluated with occurrence data from 2015. By applying optimisation procedures to select a suitable learning algorithm and climate variables that distinguish the occurrences of the species from each other at best, it was possible to achieve a high level of concordance between the short-term future forecast for the year 2015 and occurrence data from that year.

In order to represent the entire ecological niche of the species, a nested habitat model was created in a later step (Chapter 3). This model combines the results of applications of the climate model to average climatic conditions from 1981–2010 and forecasts for 2021–2050, with local landscape and wind speed data. The merging of the data with different resolutions of $1 \times 1\,\mathrm{km}^2$ (climate model results), $200 \times 200\,\mathrm{m}^2$ (wind data), and $100 \times 100\,\mathrm{m}^2$ (landscape data) was done based on fuzzy logic and the application of 48 rules. The result of this nested modelling procedure has not only a higher spatial resolution, but also performs better in validation compared to the result of the climate model for both the short-term forecast and the period from 1981 to 2010.

Finally, based on the results of the fuzzy model, an agent-based model was implemented. It simulates the regional movements of female Asian bush mosquitoes in a smaller, $625\,\mathrm{km}^2$-sized region and estimates its potential abundance and distribution hotspots over the course of the year (Chapter 4). This spatial model was linked to a dynamic infection model for WNV. The WNV model, originally developed by a research group for a region in Canada, calculates the proportions of vector mosquitoes and sentinel host birds in their respective infection stages (virus susceptible, exposed, infectious and recovered) under given temperature conditions and with a temporal resolution of one day. To apply this model to areas in Germany, parameters for population development and virus transmission were adapted to the vector mosquito species *Ae. j. japonicus* and a potential major host bird species in Europe, the Eurasian magpie *Pica pica*. The spatial model extension adds important value to the WNV model because the abundance of vector mosquitoes is an essential but mostly unknown factor in the model equations. In addition, the spatial model extension can be used to estimate the emigration of potentially infected mosquitoes

from epizootic areas to the different cardinal directions, and thus to initiate targeted mosquito control measures and inform local residents.

Zusammenfassung

Durch den internationalen Waren- und Personenverkehr haben wir ein zunehmendes Problem mit exotischen Stechmücken, die nach Europa und damit auch nach Deutschland gelangen und in vielen Gebieten invasiv werden. Die exotischen Stechmücken können die Fähigkeit zur Übertragung von Erregern für eine Reihe von Krankheiten für Menschen und andere Säugetiere mitbringen, für die es im Invasionsgebiet noch keine effizienten Überträger gibt. Außerdem können sie die Pathogene einschleppen und somit Epidemien und Epizootien auslösen. Der Klimawandel trägt dazu bei, dass sich zum einen zunehmend Arten in Mitteleuropa etablieren können, die an tropische, subtropische und mediterrane Klimata angepasst sind, und zum anderen, dass das Risiko für durch Arboviren verursachte Krankheitsausbrüche dramatisch ansteigt. Letzteres hängt unter anderem damit zusammen, dass Virusinkubationszeiten temperaturabhängig sind.

Aufgrund des zunehmenden Gefährdungspotenzials durch die exotischen Stechmücken ist es von größter Wichtigkeit, diese zu kartieren und ihre Lebensraumpräferenzen, ihr Ausbreitungsverhalten, ihre Populationsentwicklungen und ihr Vektorpotenzial zu untersuchen. Hierfür wurden das Monitoringprojekt "CuliMo" sowie das Forschungsprojekt "CuliFo" gegründet, bei denen Wissenschaftler mehrerer wissenschaftlicher Einrichtungen in Deutschland mitwirken. Durch Monitoring-Arbeiten und das Citizen-Science-Projekt „Mückenatlas" stehen zahlreiche Vorkommensdaten von Stechmückenarten in einer Datenbank bereit. Mein Ziel war die Entwicklung von Modellierungsmethoden, welche die Daten zu Artenvorkommen aus der gemeinsamen Projektdatenbank sowie biologisches und ökologisches Wissen über die invasiven Arten optimal nutzen, um ihr aktuelles und zukünftiges Ausbreitungspotenzial in Deutschland vorherzusagen.

Die Modelle wurden aufgebaut für die japanische Buschmücke (*Aedes japonicus japonicus*), die 2008 erstmals in Deutschland nachgewiesen wurde und sich seitdem rasant in vielen Teilen des Landes, ausgehend von mehreren Stellen an denen sie eingeschleppt wurde, ausgebreitet hat. *Aedes j. japonicus* ist eine mögliche Überträgerart für zahlreiche human- und tierpathogene Erreger. Unter anderem haben einige Laborversuche eine hohe Übertragungseffizienz für das West-Nil-Virus (WNV) gezeigt, welches seit 2018 auch in Deutschland in Stechmückenpopula-

tionen nachgewiesen wurde und zu Krankheitsfällen bei Vögeln, Pferden und Menschen führte. Diese Dissertationsarbeit beschreibt die schrittweise Modellierung der aktuellen und zukünftigen ökologischen Nische der Asiatischen Buschmücke in Deutschland, sowie die Generierung eines Bewegungsmodells auf der Grundlage der erstellten, detaillierten Habitatkarte, welches an ein WNV-Übertragungsmodell gekoppelt wird:

Kapitel 2 beinhaltet die Modellierung der klimatischen Nische. Hierfür wurde eine Methode entwickelt, die auch bei der Verfügbarkeit einer geringen Anzahl von Vorkommensdaten der Zielart im Invasionsgebiet angewendet werden kann. Zusätzliche Vorkommensdaten von drei heimischen Arten sowie Temperatur- und Niederschlagsdaten wurden herangezogen, um mit Hilfe maschinellen Lernens die klimatische Nische der invasiven Art von den Nischen der heimischen Arten abzugrenzen. Das Modelltraining erfolgte mit einer Support-Vektor-Maschine und Stechmückendaten der Jahre 2011 bis 2014 und die Evaluation mit Vorkommensdaten aus dem Jahr 2015. Durch den Einsatz von Optimierungsverfahren für die Auswahl eines geeigneten Lernalgorithmus und der Klimavariablen, mit deren Hilfe das Vorkommen der Arten am besten voneinander unterschieden werden kann, ist es gelungen, einen hohen Übereinstimmungswert der kurzfristigen Zukunftsprognose für das Jahr 2015 mit Funddaten aus dem Jahr zu erreichen.

Um die gesamte ökologische Nische der Art abzubilden, wurde in einem weiteren Schritt ein genestetes Habitatmodell erstellt (Kapitel 3). Dieses kombiniert die Ergebnisse von Anwendungen des Klimamodells auf klimatische Mittelwerte der Perioden 1981–2010 sowie Prognosen für 2021–2050 mit lokalen Landschafts- und Windgeschwindigkeitsdaten. Die Zusammenführung der Daten mit ihren unterschiedlichen Auflösungen von $1 \times 1\,\text{km}^2$ (Ergebnisse des Klimamodells), $200 \times 200\,\text{m}^2$ (Winddaten), sowie $100 \times 100\,\text{m}^2$ (Landschaftsdaten) erfolgte auf der Basis von Fuzzy-Logik und unter der Anwendung von 48 Regeln. Das Ergebnis dieses genesteten Modellierungsverfahrens hat nicht nur eine höhere räumliche Auflösung, sondern schneidet auch in der Validierung besser ab als die Ergebnisse des Klimamodells (sowohl bei der kurzfristigen Prognose für das Jahr 2015 als auch für die Klimaperiode 1981–2010).

Schließlich wurde auf Grundlage der Ergebnisse des Fuzzymodells ein agentenbasiertes Modell implementiert, welches die regionalen Bewegungen der weiblichen asiatischen Buschmücken in Regionen von $625\,\text{km}^2$ Größe simuliert und deren potenzielle Abundanz und Verbreitungs-Hotspots im Jahresverlauf einschätzt (Kapitel 4). Dieses räumliche Modell wurde mit einem dynamischen Infektionsmodell für das WNV gekoppelt. Das WNV-Modell, ursprünglich entwickelt von einer Forschungsgruppe für eine Region in Kanada, berechnet die Anteile an Vektormücken und Sentinel-Hostvögeln in ihren jeweiligen Infektionsstadien (krankheitsanfällig, dem Virus ausgesetzt, infektiös und genesen) unter gegebenen Temperaturbedingungen und mit

einer zeitlichen Auflösung von einem Tag. Für Modellanwendungen in Deutschland wurden die Parameter für die Populationsentwicklungen und Übertragungseigenschaften an die Vektormückenart *Ae. j. japonicus* und eine mögliche wichtige Wirtsvogelart in Europa, die Europäische Elster *Pica pica* angepasst. Die Modellerweiterung bietet einen wichtigen Mehrwert für das WNV-Modell, da die Abundanz der Überträgermücken ein wesentlicher, aber oft unbekannter Faktor in den Modellgleichungen ist. Außerdem kann auf Grundlage des Modells die Emigration potenziell infizierter Stechmücken aus den Epizootiegebieten in die unterschiedlichen Himmelsrichtungen geschätzt und somit gezielte Stechmückenbekämpfungsmaßnahmen eingeleitet und die Bevölkerung informiert werden.

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Thesis Outline

This thesis consists of 5 chapters. Chapter 1 is a general introduction that introduces the research context and outlines the objectives and scientific questions. Chapters 2 and 3 contain published articles in peer-reviewed journals adapted to the layout of this thesis. In some places, small details were added and marked accordingly. Chapter 4 includes a manuscript that is being prepared for publication in the peer-reviewed journal "Ecological Modelling" in an abbreviated form. Chapter 5 summarises and evaluates the results of the work and proposes ideas for model extensions and further applications. The following chapter overview also presents short titles for the articles. These can be found again in the headers of the respective pages and serve as orientation.

Chapter 1
Introduction

Chapter 2
Short title: Modelling the climatic niche
Long title: Can data from native mosquitoes support determining invasive species habitats? Modelling the climatic niche of *Aedes japonicus japonicus* (Diptera, Culicidae) in Germany

Chapter 3
Short title: Nested modelling approach based on fuzzy set theory
Long title: What makes the Asian bush mosquito *Aedes japonicus japonicus* feel comfortable in Germany? A fuzzy modelling approach

Chapter 4
Short title: West Nile virus model with mosquito flight simulator
Long title: Linking a compartment model for West Nile virus with a flight simulator for vector mosquitoes

Chapter 5
General Discussion

List of Publications with Author Contributions

Kerkow, Antje, Ralf Wieland, Marcel B. Koban, Franz Hölker, Jonathan M. Jeschke, Doreen Werner, and Helge Kampen. What makes the Asian bush mosquito *Aedes japonicus japonicus* feel comfortable in Germany? A fuzzy modelling approach. *Parasites & Vectors* 12, no. 1 (14 March 2019): 106. https://doi.org/10.1186/s13071-019-3368-0.

AK and RW were responsible for the conception and design of the model. AK did the literature research, the acquisition, analysis and interpretation of data and drafted the manuscript. MK, DW and HK contributed biological expert knowledge. RW, HK, FH, JMJ and MK critically revised the manuscript. All authors read and approved the final manuscript.

Kerkow, Antje, Ralf Wieland, Linus Früh, Franz Hölker, Jonathan M. Jeschke, Doreen Werner, and Helge Kampen. Can data from native mosquitoes support determining invasive species habitats? Modelling the climatic niche of *Aedes japonicus japonicus* (Diptera, Culicidae) in Germany. *Parasitology Research*, 119, no. 1 (1 January 2020): 31–42. https://doi.org/10.1007/s00436-019-06513-5.

AK, RW and LF were responsible for the conception and design of the model. AK and LF conducted the literature research. AK analysed and interpreted the model results and drafted the manuscript. DW contributed biological expert knowledge. RW, HK, JMJ, FH and LF critically revised the manuscript. All authors read and approved the final manuscript.

In preparation: **Kerkow, Antje**, Ralf Wieland, Jörn M. Gethmann, Franz Hölker, and Hartmut H. K. Lentz. Linking a compartment model for West Nile virus with a flight simulator for vector mosquitoes. Submitted to *Ecological Modelling* in a shortened form.

AK was responsible for the conception of the study and conducted the literature research. AK and RW designed the model. HHKL supported the data analyses. AK analysed and interpreted

the model results and drafted the manuscript, JG contributed parasitological and epidemiological expert knowledge. FH contributed expert knowledge for the conception of agent based models. RW, JG and HHKL critically revised the manuscript.

Chapter 1

General Introduction

1.1 Mosquitoes: Medical relevance and risks posed by exotic species in Europe

Mosquitoes (Culicidae) are one of seven families within the order of Diptera. Around 3,200 to 3,500 species of mosquitoes exist worldwide, of which approximately 90 have been detected in Europe, and 50 in Germany [1–3]. The hematophagous feeding behaviour of the females ranks mosquitoes first in terms of medical relevance among arthropods, closely followed by ticks [4]. Species of the genus *Anopheles* transmit plasmodia causing malaria, a disease estimated to have killed between 1,200,000 and 1,800,000 people annually worldwide between 2004 and 2010 [5]. Furthermore, filarial nematodes and about 300 types of viruses are transmitted by mosquitoes, of which about 100 infect humans [6]. In addition to the risk of pathogen transmission, massive attacks and bites by female mosquitoes can lead to serious blood loss, cause allergic reactions due to the transmitted proteins, and negatively affect the feeding behaviour of livestock and other animals [6].

Since the eradication of malaria in Europe in the 1960s, mosquito-borne diseases and related research have had little relevance on the continent [3, 7, 8]. In Germany, studies of the mosquito fauna concentrated on areas with nuisance potential [3]. The research situation has changed fundamentally as of the turn of the millennium, when exotic mosquito species of the genus *Aedes*, some of them with great vector potential for human pathogens, were frequently introduced (and re-introduced). To date, four species of medical relevance have become established on the continent: the yellow fever mosquito *Aedes aegypti* (Linnaeus 1762), the Asian tiger mosquito *Aedes albopictus* (Skuse 1894), the Asian bush mosquito *Aedes japonicus japonicus* (Theobald

1901) and the Korean bush mosquito *Aedes koreicus* (Edwards 1907) (Tab. 1.1). Other mosquito species that have been imported since have failed to establish successfully. These include, for example, the American rock pool mosquito *Aedes atropalpus* (Coquillett 1902) and the American treehole mosquito *Aedes triseriatus* (Say 1823) [9, 10].

The thermophilic species *Ae. aegypti* and *Ae. albopictus* are the main vectors of several pathogens that pose a major health risk to humans, including Zika, chikungunya, dengue and yellow fever virus (Tab. 1.1) [6, 10–12]. The danger posed by the presence of these species became evident through disease outbreaks after its establishment in the respective regions of Europe, already in the following years of its establishment. Following the introduction of *Ae. albopictus*, there was a chicungunya epidemic in Italy in 2007, and occasional confirmed autochthonous (non-imported) human cases of chicungunya virus transmission in 2010 and 2014 [10, 13, 14]. Moreover, autochthonous dengue virus transmission occurred in France and Croatia in regions where *Ae. albopictus* was detected, for the first time in Europe since the 1920s. On the island of Madeira (Portugal), there was a large dengue virus outbreak in 2012/13 caused by *Ae. aegypti*, which has been re-introduced to the island since 2005 [10, 14].

Climate change contributes to the fact that, on the one hand, species such as *Ae. aegypti* and *Ae. albopictus*, which are adapted to tropical, subtropical and Mediterranean climates, can establish themselves to an increasing extent in Central Europe and, on the other hand, that the risk of arbovirus-related disease outbreaks increases dramatically. The increased risk for epidemics and epizootics is due to the exponential dependence of the extrinsic incubation period (the incubation time in the mosquito) on the ambient temperature [12, 25]. Temperature also affects the rate of protein digestion in the female mosquitoes, shortening the gonotrophic cycle, i.e. the time from taking blood to oviposition, so that blood hosts are visited more frequently [6, 26].

The main cause of the frequent introduction of exotic mosquitoes is the vastly increased worldwide trade in goods and travel. As early as the 16th century, when the intercontinental trade in goods and slaves flourished due to seafaring, *Ae. aegypti* and, along with it, the yellow fever virus were spread from Africa to North and South America and the Caribbean [6, p. 289-290]. In this historical scenario, both infected hosts and vectors were transported simultanuously, and oviposition sites for the mosquitoes were available in the form of water containers [27]. However, as numerous examples demonstrate, the simultaneous introduction of vector and pathogen is not necessary to create a risk scenario. Due to the high mobility of people and the intensity of flight traffic today, human pathogens can easily be introduced retrospectively into the established vector mosquito populations. It is also possible that pathogens for which the introduced mosquito species is an efficient vector are already present in the invasion area. However, until then, they were only

Table 1.1: Overview of the established invasive mosquito species in Europe

Species	Origin	Year, country of first detection in the EU	Year of first detection in Germany	Disease agents transmitted[1]	Further vector competence[2]
Ae. aegypti (Linnaeus 1762)	Islands of the SW-Indian Ocean, Afrika [16]	2004, Madeira/ Portugal (re-introduced) [10]	2016 (not established) [15]	CHICV, DENV, YFV [14]; ZIKV [12]	VEEV [14]; WNV [10]
Ae. albopictus (Skuse 1894)	SE-Asia [17]	1979, Albanien (spread in Europe since 1990) [10]	2007 [3]	CHICV, DENV, DIRO [14]; ZIKV [12]	EEEV, JCV, JEV, LACV, VEEV, WNV [14]; AEFV, CVV, MYV, OPV, POTV, RRV, RVFV, SAV, SINV, TVV, USUV, WEEV, YFV [10];
Ae. j. japonicus (Theobald 1901)	Korea, Japan, Taiwan, S-China, SE-Russia [18]	2000, France [10]	2008 [3]		CHIKV, DENV, EEEV, JEV, LACV, SLEV, WNV [14]; RVFV [10]; CVV [19]; ZIKV [20–22]; USUV [21]; DIRO [23]
Ae. koreicus (Edwards 1917)	Japan, NE-China, S-Korea, Russia [24]	2008, Belgium [10]	2015 [3]		JEV, DIRO [14]

[1] : refers to pathogens for which the mosquito species is a proven key vector in the field, [2] : refers to pathogens that have been isolated from field-collected specimens and/ or for which potential transmission has been demonstrated in the laboratory; Pathogen abbreviations: AEFV = insect-infective *Aedes* flavivirus, CHICV = Chikungunya Virus, CVV = Cache Valley virus, DENV = Dengue virus, DIRO = *Dirofilaria immitis*, *Dirofilaria repens*, EEEV = Eastern equine encephalitis virus, JCV = Jamestown Canyon virus, JEV = Japanese encephalitis virus, LACV = La Crosse virus, MYV = Mayaro virus, OPV = Oropouche virus, POTV = Potosi virus, RRV = Ross River virus, RVFV = Rift Valley fever virus, SAV = San Angelo virus, SINV = Sindbis, SLEV = Saint Louis encephalitis, TVV = Trivittatus virus, USUV = Usutu virus, VEEV = Venezuelan equine encephalitis virus, WEEV = Western equine encephalitis virus, WNV = West Nile virus, YFV = Yellow fever virus, ZIKV= Zika virus

transmitted by less efficient vectors, so epidemics did not occur until the time of introduction of the more efficient vector [27]. A prominent example is the introduction of *Anopheles gambiae* into Brazil, which led to malaria epidemics caused by the previously resident pathogen *Plasmodium falciparum* [27].

The biggest culprits for transporting mosquitoes between continents today are cargo containers from ships containing used tyres or ornamental plants such as lucky bamboo [28]. Lucky bamboo

has been transported on a large scale from China to the Netherlands and USA, either directly in stagnant water or as stems placed in water upon arrival, and has led to the mass introduction of *Ae. albopictus* [28–31]. Standing water provides a habitat for larvae, greatly increasing the risk of mosquito introduction, but species of the genus *Aedes* are not necessarily dependent on it. The Aedini are characterised by desiccation-resistant eggs that are not laid directly on a water surface as in species of most other genera, but on moist substrate. Embedded in the egg, the embryos survive in quiescence until exposed to a hatching stimulus [2, p. 10-11], [6, 32, 33]. Some species also have an embryonic or pre-embryonic, photoperiodic diapause, for example *Ae. j. japonicus* and populations of *Ae. albopictus* in temperate regions. The (pre-)diapause facilitates hibernation in regions with frost as well as the long-distance transport of mosquitoes [32–35].

Besides transport in container ships, adult mosquitoes have often been found in passenger cabins of airplanes. Mosquito transport by airplanes is the reason for the phenomenon of so-called "airport malaria" [28]. Intercontinentally, invasive *Aedes* species spread partly by active flight and partly by hitch-hiking along roads [31, 36–39].

1.2 Mosquito monitoring measures in Europe

In response to the spread of exotic mosquito species and the increasing risk potential for arboviruses, numerous European countries have initiated monitoring and research projects to study the exotic, but also the native mosquito species and the pathogens they may transmit. In addition to classical monitoring methods conducted by biologists, some citizen-supported projects, so-called "citizen science" projects, have been established and promoted by the media (Tab.1.2). Depending on the programme, volunteers participate by either collecting mosquitoes and sending them to the respective coordination team for identification, uploading photos via a smartphone application to help identify the species, and/or filling out an online questionnaire to assess the local mosquito densities and their nuisance potential [40–42].

In contrast to classical monitoring programmes, citizen-based monitoring is relatively inexpensive and, if the intensity of use is maintained at a high level, enables monitoring over a large area and a close temporal coverage [40, 41, 43]. Citizen science projects for mapping mosquito diversity, such as the "Mückenatlas" in Germany and "Mosquito Alert" in Spain, have been shown to be good early warning systems for the emergence of exotic species close to human settlements, as well as for jumps in dispersal within the country to remote locations [41, 44–46].

Table 1.2: Citizen science programmes for studying the mosquito fauna in Europe [40–42]

Country	Name	Submissions
France	iMoustique	photograph via smart-phone application
Germany	Mückenatlas	physically via mail
Italy	ZanzaMapp	online questionnaire
Portugal	MosqutoWEB	physically via mail
Spain	Mosquito Alert (former "Atrapa el Tigre")	photograph via smart-phone application
The Netherlands	Muggenradar	physically via mail
UK	Mosquito Reporting Scheme	physically via mail

1.2.1   Recent mosquito monitoring activities in Germany

In Germany, a programme for culicid monitoring was developed and launched in 2011 and 2012, combining passive (the Mückenatlas project) and active approaches [44]. In the passive approach, citizens have been encouraged since 2012 to collect adult mosquitoes from any location within Germany, freeze them in a small container for killing, and then send them in the container by post to a participating institute for species identification (with the postage paid by the sender) [47, 48]. Already in the first year, more than 6,000 mosquitoes from all over Germany were submitted, and by the end of 2019, more than 137,000 [3, 48].

The active monitoring involved biologists and scientific staff systematically collecting mosquito larvae by dipping, and imagoes by trapping, netting and aspirating from 2011 to the end of 2017. BG Sentinels and EVS traps were partly used for 24h per week during the period from April to October at more than 100 different locations throughout the country [3, 44]. Larval dipping was used intensively at sites of invasive species submitted to the Mückenatlas to verify and investigate new local occurrences and the subsequent spread of the species [45].

1.3   Objectives of this work

The aim of my work was to develop modelling methods that make optimal use of the available species occurrence data from Germany's extensive culicide database "CulBase", which contains both the active and passive monitoring data, to predict the potential distribution range of mosquito species. A key issue was to find a way that does not rely exclusively on machine learning methods and species occurrence data in combination with environmental variables, but also allows for the integration of biological expertise. This is important to compensate for quality weaknesses in the

data in the form of unintended bias that arises from passive monitoring. The biggest problem with this type of monitoring is that the submissions are mainly derived from densely populated regions and thus do not cover all regions, types of land use and (micro-) climatic zones of Germany equally well [46].

It has been of greatest interest to predict the possible future distribution of the Asian bush mosquito (*Aedes japonicus japonicus*), sometimes also referred to as the Asian or Japanese rock pool mosquito, in Germany. The Asian bush mosquito was first recorded as an invasive species in Europe in 2003, and then in Germany at the border with Switzerland in 2008 [49, 50]. The species is native to Southeast Asia (Korea, Japan, Taiwan, South China and Southeast Russia) and has become the most widespread and ambundant invasive mosquito species in Germany [18, 51]. Due to its adaptation to temperate climates, *Ae. j. japonicus* was able to spread and become permanently established in Central Europe, probably starting from several locations where it was introduced (Fig. 1.1). It must be assumed that the populations in Europe can no longer be completely eradicated [52].

The Asian bush mosquito is currently not known to be a primary vector of disease-agents in the field, though it has been confirmed to be a competent vector for numerous arboviruses and filarial nematodes in the laboratory (Tab. 1.1), [40]. For West Nile virus (WNV), even very high dissemination rates were detected in some experiments [54–57]. In addition, WNV and Japanese encephalitis virus were found in individuals collected in the field, indicating possible vector competence. WNV has emerged in Germany for the first time in 2018 and now apparently also persists in native mosquito species during winter [58–61]. The arbovirus is transmitted between mosquitoes and birds, but humans can also become infected; they are so-called dead-end hosts for the virus (see Chapter 4 for more information on the WNV and Fig. 1.2 for the current distribution in Europe).

The selection of *Ae. j. japonicus* as a model species was based on its medical relevance as well as its high abundance and the presence of a large amount of occurrence data in the database. Sufficient occurrence data are important preconditions for the application of modelling techniques driven by occurrence data. The aim was to fundamentally establish the methods in order to be able to subsequently apply them to other invasive as well as native species of interest.

In the following chapters, this dissertation describes the development of a procedure for modelling the current and future (realised) ecological niche of the invasive Asian bush mosquito in Germany in several steps. Chapter 2 covers the modelling process of the large scale climatic niche with the help of a supervised classification algorithm (Support Vector Machine). The elaboration of the basic method of separating climate data at the occurrence sites of different
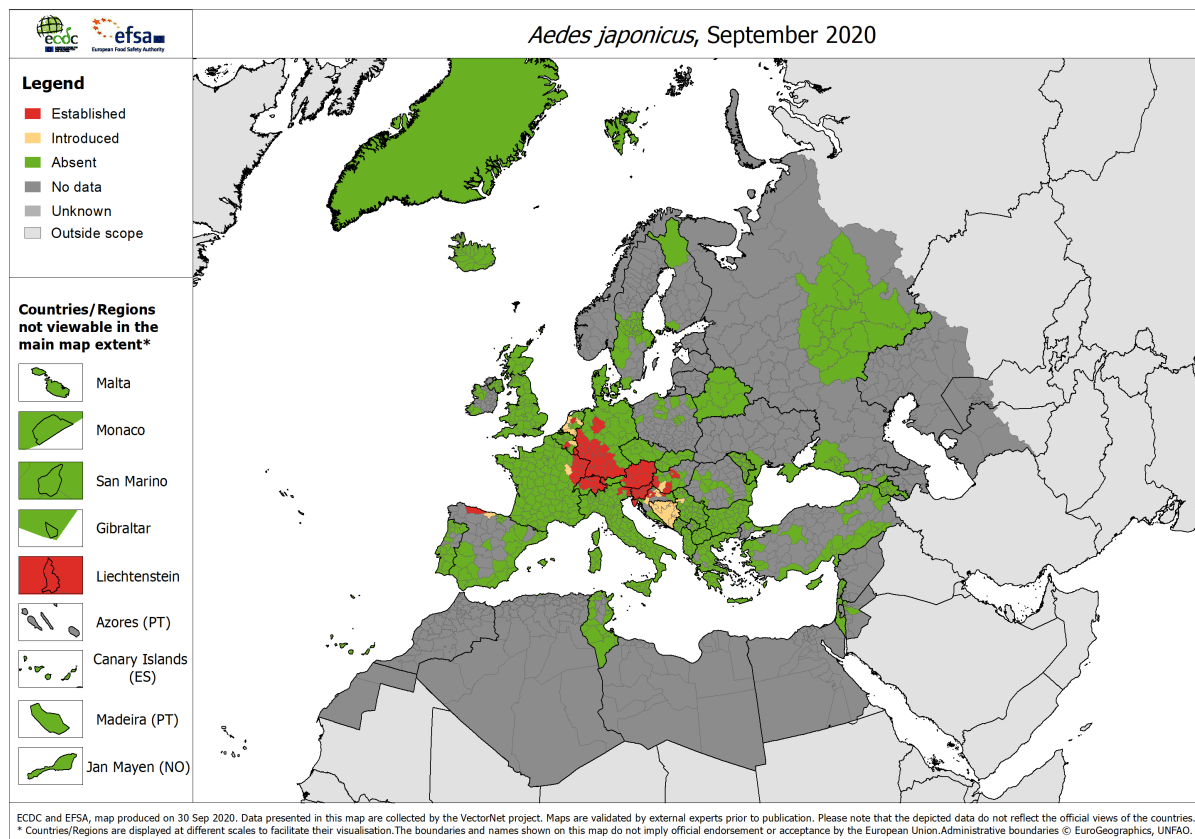
Figure 1.1: Current known distribution of *Aedes japonicus japonicus* in Europe at the 30th of September 2020 [53].

mosquito species, and thereby analysing the individual niche of the target species, is described. Chapter 3 presents the modelling of the ecological niche on a local scale by logically linking vegetation and land use as well as wind speed data to the output of the climate model while incorporating expert biological knowledge. In Chapter 4, I demonstrate how the detailed habitat map of the invasive mosquito species can be used to implement a model for predicting local abundances and dispersal of mosquitoes within a region of a few hectares over the course of a year. This so-called mosquito flight simulator was coupled to a dynamic compartment model for West Nile virus transmission and can also illustrate the number and possible positions and flight routes of infected mosquitoes. Based on the West Nile virus model, I also compare in Chapter 4 the vector capacities of *Ae. j. japonicus* with that of a *Culex* species aggregate, thus giving an outlook on the vector potential of the species from a modelling point of view.

Distribution of West Nile virus infections in humans by affected areas in the EU/EEA countries and EU neighbouring countries
Transmission season 2020 and previous transmission seasons; latest data update 26 Nov 2020

Human cases reported in 2020
Human cases reported in 2019
Human cases reported during 2011–2018
No reported cases
Not included

ECDC. Map produced on: 27 Nov 2020

Figure 1.2: Human West Nile virus cases reportet between 2011 and 2020 in Europe [62]

1.3.1 Research questions

In summary, the leading questions of my work were:

(i) How can the climatic and overall ecological niche of a relatively recently established invasive mosquito species be predicted using only presence data? Can presence data of other mosquito species help to predict the potential spread of the target species?

(ii) Can the accuracy of the model be improved with the help of (also vague) expert knowledge and other factors than climate, which undoubtedly plays a major role for exothermic insects?

(iii) Where are the suitable distribution areas of the Asian bush mosquito *Aedes japonicus japonicus* in Germany under current and future climate conditions and regarding different types of land use?

(iv) Where are regional hotspots, dispersal corridors and stepping stones of *Aedes japonicus japonicus* potentially infected, e.g. with West Nile virus?

(v) Asian bush mosquito versus common house mosquito (*Culex pipiens agg.*): Who is more efficient in initiating and maintaining a West Nile virus transmission cycle between mosquitoes and birds?

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Chapter 2

Modelling the climatic niche

Paper title: Can data from native mosquitoes support determining invasive species habitats? Modelling the climatic niche of *Aedes japonicus japonicus* (Diptera, Culicidae) in Germany

Authors   Antje Kerkow[a,b], Ralf Wieland[a], Linus Früh[a], Franz Hölker[b,c], Jonathan M. Jeschke[b,c,d], Doreen Werner[a], Helge Kampen[e]

a   Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany
b   Freie Universität Berlin, Department of Biology, Chemistry, Pharmacy, Institute of Biology, Königin-Luise-Str. 1-3, 14195 Berlin, Germany
c   Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany
d   Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstr. 34, 14195 Berlin, Germany
e   Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald - Insel Riems, Germany

2.1 Abstract

Invasive mosquito species and the pathogens they transmit represent a serious health risk to both humans and animals. Thus, predictions on their potential geographic distribution are urgently needed. In the case of a recently invaded region, only a small number of occurrence data is typically available for analysis, and absence data are not reliable. To overcome this problem, we have tested whether it is possible to determine the climatic ecological niche of an invasive mosquito species by using both the occurrence data of other, native species and machine learning. The approach is based on a support vector machine and in this scenario applied to the Asian bush mosquito (*Aedes japonicus japonicus*) in Germany. Presence data for this species (recorded in Germany since 2008) as well as for three native mosquito species were used to model the potential distribution of the invasive species. We trained the model with data collected from 2011 to 2014 and compared our predicted occurrence probabilities for 2015 with observations found in the field throughout 2015 to evaluate our approach. The prediction map showed a high degree of concordance with the field data. We applied the model to medium climate conditions at an early stage of the invasion (2011–2015), and developed an explanation for declining population densities in an area in northern Germany. In addition to the already known distribution areas, our model also indicates a possible spread to Saarland, southwestern Rhineland-Palatinate and in 2015 to southern Bavaria, where the species is now being increasingly detected. However, there is also evidence that the possible distribution area under the mean climate conditions was underestimated.

2.2 Introduction

Due to globalisation, facilitating long-distance traffic, mass tourism and worldwide trade, increasing numbers of invasive mosquitoes have recently arrived and subsequently established themselves in Germany and mainland Europe (Medlock et al. 2015). As they include potential vectors of a wide range of human and animal pathogens (Schaffner et al. 2013; Becker et al. 2014), they have become a major research issue. To estimate the risk and take protective measures against mosquito-borne disease outbreaks, it is of utmost importance to know the suitable habitats of the various vector species.

We think that the habitats of invasive species can be analysed most effectively in a stepwise procedure. The first step should be to identify the climatic niche within the invaded area. This is challenging because the spread of invasive species (especially short-lived exothermic insects) is

influenced by spontaneous weather events on the one hand, but also by long-term climate, which has impacted vegetation and mosquito populations in the past. In the second step, landscape elements such as land use and altitude need to be considered. However, some regions, although suitable for an invasive species, are unlikely to become populated by active migration due to dispersal barriers. In the case of mosquitoes, for example, these can be large cultivated areas. On the other hand, there are propagation paths that enable rapid passive spread, for example, along roads and waterways, because some mosquito species may be displaced by cars in their adult forms (Eritja et al. 2017) or by container ships in their egg stages (Eritja et al. 2005; Hofhuis et al. 2008; Reiter 2010). Therefore, in the third step, the consideration of propagation paths is useful, including a propagation simulation. In this paper, we focus on the first step and try to identify climatically suitable areas for the Asian bush mosquito (*Aedes japonicus japonicus* (Theobald, 1901)) in Germany.

The species is one of eight non-indigenous culicid species recently registered in Germany, and apparently the most wide-spread of them (Kampen et al. 2017; Koban et al. 2019). It originates from East Asia (Miyagi 1971) and was first recorded in 1998 and 2000 in North America and Europe. Since then, it has expanded its distribution range rapidly on both continents (Peyton et al. 1999; Kampen and Werner 2014; Kampen et al. 2017). In its native range as well as in most invasion areas, the climate is temperate and characterised by winters with frost and snowfall. However, it was also found in subtropical and tropical climates such as Florida and Hawaii (Egizi and Fonseca 2015; Riles et al. 2017). The eggs of this species are desiccation and frost resistant (Reuss et al. 2018) and are laid by the females in rock pools of rivers, water-filled tree holes or various kinds of small artificial containers that are able to collect water such as flower pots or vases, buckets, ash trays and bird baths (Tanaka et al. 1979; Scott 2003; Kampen et al. 2012; Kaufman et al. 2012). Egg hatching and larval development in spring begin at 4–5°C, and development time decreases significantly with temperatures rising up to 28°C (Scott 2003; Burger and Davis 2008; Kampen et al. 2016a). Temperatures above 34°C inhibit larval development (Scott 2003). The species usually overwinters in the egg stage, but in warmer regions, it is also possible and observed to hibernate in the larval stage (Reuss et al. 2018; Bova et al. 2019). Immature stages are usually found both sooner in spring and later in autumn than coexisting mosquito species (Iriarte et al. 1991; Burger and Davis 2008; Kaufman and Fonseca 2014).

For habitat analysis of both invasive and native species, data-driven machine learning approaches have been widely applied and proven successful (Drake et al. 2006; Jeschke and Strayer 2008; Früh et al. 2018). In general, species distribution models can be created using either presence-only or combined presence-absence data. Absence data would doubtlessly improve

the distribution predictions for an invasive species (Elith et al. 2006; Vaclavik and Meentemeyer 2009), and they can be obtained either via monitoring programmes or computer simulations. The simulated so-called background or pseudo-absence data are randomly distributed points in the entire model area or only in regions without evidence and with a certain spatial distance to the known occurrences (VanDerWal et al. 2009; Barbet-Massin et al. 2012).

When modelling invasive species that have only recently been established, the available dataset often poses a problem because usually only a small number of presence data are available. Furthermore, absence data cannot be used for modelling (Liu et al. 2013), as it is unknown whether the species is not present in a certain area because *(i)* the area does not offer appropriate habitat conditions, *(ii)* the area is not accessible due to dispersal barriers, or *(iii)* not enough time has passed for the species to arrive. Additionally *(iv)*, it is possible that the species does occur in an area, even though it has not been found during field surveys. The latter may particularly be the case when very large areas are surveyed, as during the *Ae. j. japonicus* monitoring throughout Germany (Kampen et al. 2016a).

Although the use of background/pseudo-absence data leads to an artificially enforced high evaluation of a model and is therefore hotly debated (VanDerWal et al. 2009; Vaclavik and Meentemeyer 2009), the majority of invasive mosquito distribution models rely on them. Currently, the most frequently applied system of modelling is the maximum entropy (MaxEnt) modelling algorithm (Phillips et al. 2006; Fischer et al. 2011; Rochlin et al. 2013; Thomas et al. 2014; Melaun et al. 2015). Combinations of machine learning algorithms for presence and background data have also been used with the objective to obtain more robust predictions than with a single algorithm (Kraemer et al. 2015; Cunze et al. 2016). However, this procedure is also under discussion (Demertzis et al. 2017).

Predictive distribution maps based on either single or combined machine learning algorithms risk underestimating the future habitats of invasive species, as only a short time has passed since the arrival of the species. Furthermore, training data may not reflect all possible ranges under the selected climate variables due to lacking propagation paths between the suitable habitats. To circumvent these problems, scientists have used data from other countries and continents, where the mosquito species of interest is native or had already been present for a longer period of time (Fischer et al. 2011; Thomas et al. 2014). In this way, a large dataset can be provided. However, a given species may fill specific ecological niches in particular countries due to different environmental contexts (Jeschke and Strayer 2008). Therefore, using data of the native range may not always lead to accurate predictions for an invaded area.

Thanks to a nationwide monitoring programme established in Germany several years ago, which also includes the citizen science project "Mückenatlas" (Werner et al. 2014; Kampen et al. 2015; Walther and Kampen 2017), a large amount of occurrence data is available concerning the German mosquito fauna, which includes around 50 species. Based on the idea that each species probably occupies its individual and unique ecological niche, we have tested whether it is possible to distinguish the climatic niche of *Ae. j. japonicus* from the climatic niches of native species. Thus, contrary to modelling approaches relying on occurrence data outside the model region, as well as to a modelling approach based on life history data obtained from laboratory analyses (Wieser et al. 2019), we aim to determine the realised ecological niche (Jeschke and Strayer 2008). This was achieved by the application of a machine learning approach based on a support vector machine (Cortes and Vapnik 1995), presence data of *Ae. j. japonicus* and three native mosquito species from the invaded region (Germany). The algorithm calculates a dividing hyperplane for the vector dataset of environmental parameters and for the four mosquito species, respectively. It is not easy to identify which weather data explain the distribution patterns of the species at best. We have therefore developed a procedure in the framework of this study, which has been published separately (Wieland et al. 2017).

The support vector machine (Cortes and Vapnik 1995) is a supervised learning algorithm and frequently used for data-driven species distribution models (e.g. Drake et al. 2006; Fukuda et al. 2013). It was developed in the 1990s along with a number of other machine learning techniques such as regression tree and random forest. The algorithm was primarily implemented as a linear binary classifier that maximises the margin between two sampling groups (Cortes and Vapnik 1995; Kampichler et al. 2010). When calculating the exact position and orientation of the hyperplane that separates the groups from each other, the algorithm does not consider all data points, but only those closest to the plane, the so-called supported vectors. Advanced forms of support vector machines use non-linear kernels (Nalepa and Kawulok 2019). By means of the "kernel-trick", the training data are mapped into a higher dimensional space which simplifies the computation of the separating hyperplanes for complicated patterns (Drake et al. 2006; Kampichler et al. 2010; Nalepa and Kawulok 2019). A kernel of the RBF (radial basis function) type was used in this approach.

Research by our working group (Kerkow et al. 2020[1], Früh et al. 2018) has shown that even with noisy data, the application of the support vector machine in combination with a low-dimensional space-kernel is relatively robust against over-fitting. The algorithm is thus a suitable method of machine learning for our relatively small data sets which are prone to this issue. Früh

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[1]This reference has been added and is not included in the published version of this article.

et al. (2018) found that the algorithm did not always achieve the highest accuracy in training, but often came out best in validation. The support vector machine as a classifier produces binary outputs, but probability values can be generated in a post-processing step (Platt 1999). These probabilities give a much more detailed picture of the spatial extent of the habitat than binary values.

In summary, the aims of this study are *(i)* to develop a model dependant on climate parameters that enables accurate predictions of future distributions of an invasive mosquito species based on a limited amount of presence-only data, and *(ii)* to apply this model to *Ae. j. japonicus* in Germany.

2.3   Materials and methods

2.3.1   Native species

For habitat separation, we have selected two native mosquito species that are widespread and common throughout Germany: *Aedes vexans* (Meigen, 1830) and *Aedes geniculatus* (Olivier, 1791). *Aedes vexans* is a floodwater species of the lowlands, where it develops in temporary water bodies. It is adapted to temperate climates (Becker et al. 2010) and develops in tremendous numbers after heavy rainfall and flooding. Larval hatching starts at temperatures above $9.5°C$, but development is optimal at $30.5°C$. As with *Ae. j. japonicus*, the eggs are resistant against drought and frost and can even sustain temperatures as low as $-20.5°C$ (Becker et al. 2010).

Aedes geniculatus preferably breeds in water-filled tree holes, open tree stumps and branch axils that can be found on mature, deciduous trees (Dahl and Blackmore 2001). Eggs are laid on humid walls of the wood, and the larvae hatch after rainwater has filled the holes (Dahl and Blackmore 2001). Like the Asian bush mosquito, *Aedes geniculatus* overwinters either in the egg stage in cold regions or in the larval stage in warmer regions and the eggs can withstand freezing.

We additionally picked one species, *Anopheles daciae* (Linton, Nicolescu and Harbach 2004), whose occurrence is particularly concentrated in the north-eastern part of Germany in order to take account of particular continental conditions in Germany. *Anopheles daciae* is a member of the *Anopheles maculipennis* complex, which has only recently been described (Nicolescu et al. 2004). Little is known yet about the ecology of this species. In the framework of a nationwide mosquito monitoring programme in Germany, *An. daciae* was found in the northern Upper Rhine Valley, in the eastern North German lowlands and in the lower river valleys in southern Germany (Kampen et al. 2016b). This indicates linkage of occurrence to both low altitude and relatively continental climate conditions.

Figure 2.1 shows the collection sites of each model species in Germany from 2011 to 2014, based on nationwide systematic active and passive mosquito monitoring. The passive monitoring data originate from the "Mückenatlas" project and account for 68.9 % of the data used for training and evaluation of this model. The active monitoring data are derived from on-site collections in regions from which invasive mosquito species were submitted, by examining possible breeding sites for larvae and installing mosquito traps.
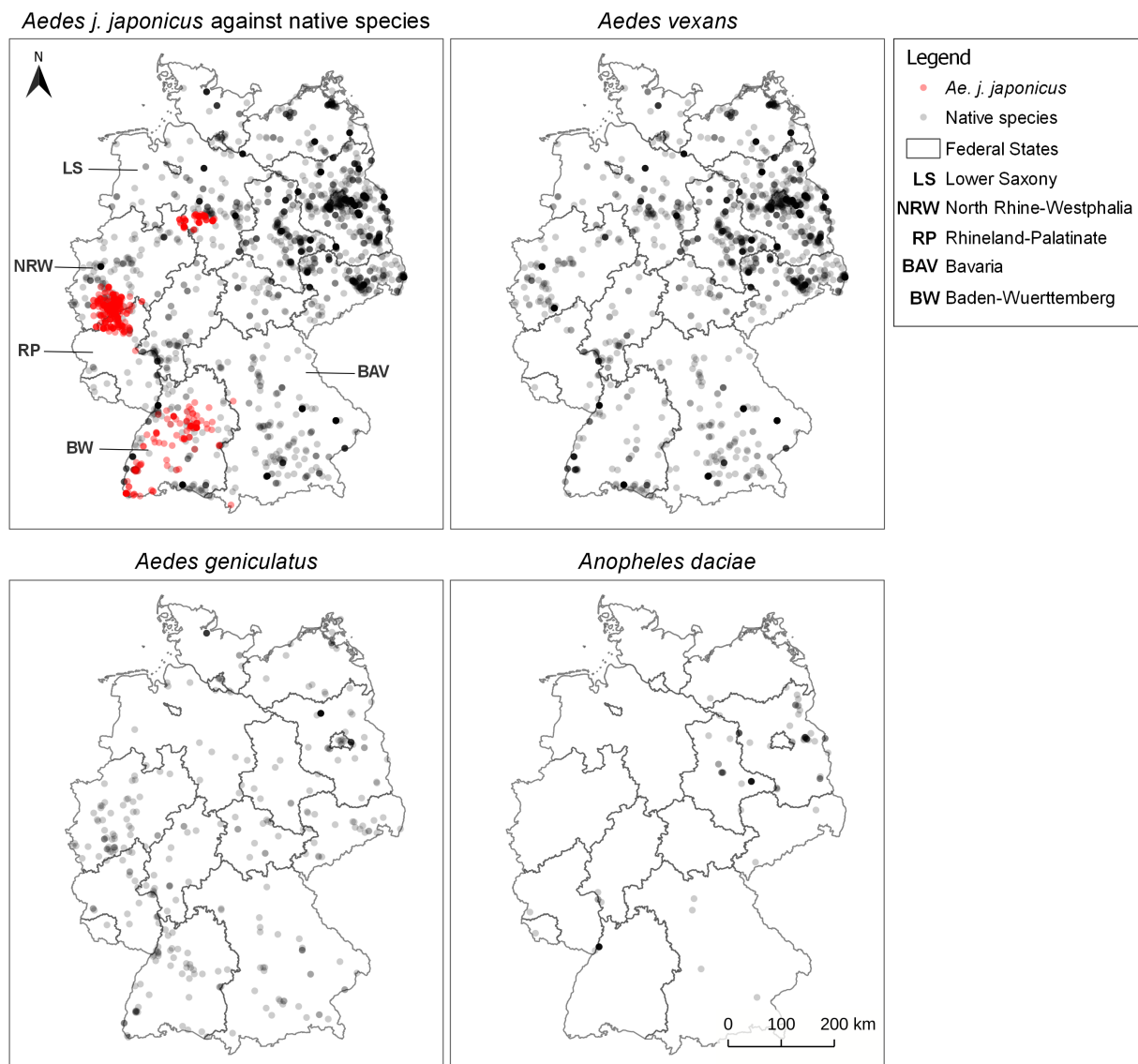
Figure 2.1: Visualisation of species sampling data of the years 2011–2014 (training years for the model). The evidence points appear in high colour intensity when several of the same colour overlay each other. This image was created with QGIS 3.8.2

2.3.2 Data access

Mosquito database

The mosquito occurrence data are derived from monitoring projects carried out in Germany since 2011 and stored inside the German national mosquito database (CULBASE, https://culbase.fli. de/Public/Page/Info.aspx). Amongst others, the database contains collection sites (including coordinates), mode of collection (including dates) and methods of species determination. The CULBASE database can be accessed via an export interface that enables querying data of the species and the year of interest, and downloading these data as Excel files, one file for each species. Data of *Ae. j. japonicus*, *Ae. vexans*, *Ae. geniculatus* and *An. daciae* from the years 2011–2014 were filtered for training the model, and data from 2015 to evaluate it. The Excel files contained the coordinates and the dates of collection. These data were entered into our object-oriented model using the PYTHON "PANDAS" module.

Weather and climate data

We used freely available data from the German Weather Service (Deutscher Wetterdienst 2017) which are gridded for Germany with a cell size of 1 km × 1 km and provide hourly, daily, monthly, seasonal, yearly and multi-annual resolutions. In the following, we define data up to an annual resolution as weather data and multi-year averages as climate data.

For each of the four mosquito species and their occurrence points in the training years 2011, 2012, 2013 and 2014, we created Excel tables with the help of a PYTHON script. These tables were the input for training the model; they contained the weather data for each occurrence point. According to Wieland et al. (2017), we used the following eight weather variables: mean temperature in spring (March, April, May) [T13], September [T09], October [T10] and December [T12]; sum of precipitation in February [P02], April [P04] and June [P06]; and drought index of autumn (September, October, November) [D15]. Wieland et al. (2017) use the same model as presented here and describe a method for the analysis of weather data that explain the distribution of the mosquito species at best. Therefore, a set of 37 data was pre-selected that may play a role in a biological point of view. These were, for example, temperature and precipitation data during the breeding season and the temperature and number of frost days during the winter months. The data sets were reduced to the best combination using a genetic optimisation procedure. The optimisation parameter was the *f1* score (see subsection "Model validation" (2.3.4) for the definition).

2.3.3   Model training

As shown in Fig. 2.2, we used the species occurrence data and the weather data from 2011 to 2014, respectively, for training the model. The support vector machine only needed to distinguish between two classes, the invasive target species on the one hand, and the native species on the other hand. By combining the monitoring data of several native species to one class, we converted a multi-classification problem into a binary classification problem, which helps to correctly calculate a decision boundary for the target species in space (Garreta and Moncecchi 2013).
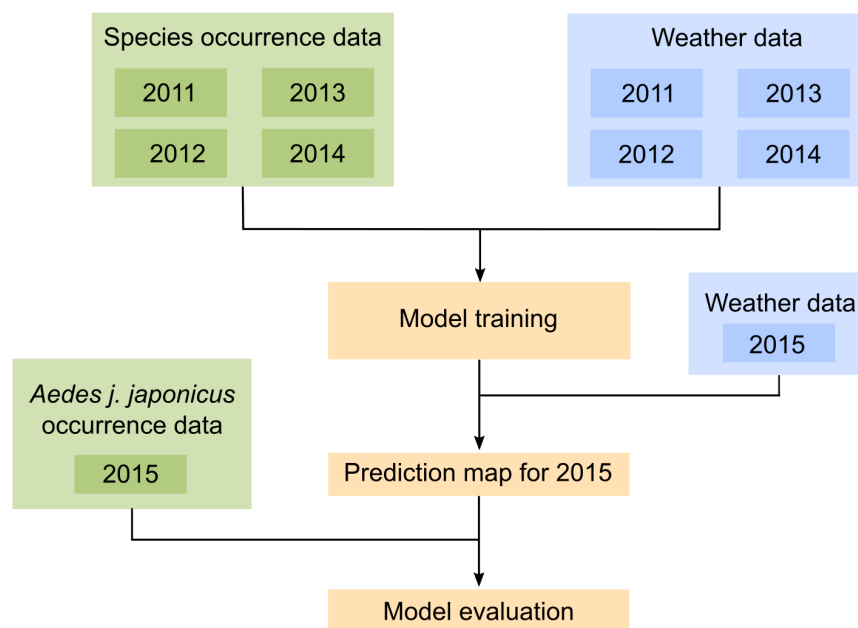
Figure 2.2: Workflow: Model training and validation. This image was created with Inkscape 0.92

As a training algorithm, we used the support vector machine from the SCIKIT toolbox (Pedregosa et al. 2011). Our training dataset contained 508 occurrence points for *Ae. j. japonicus*, 2056 for *Ae. vexans*, 322 for *Ae. geniculatus* and 102 for *An. daciae*. These data and the stored weather conditions were read in from the Excel tables with the PYTHON "PANDAS" tool. To mitigate the unbalanced numbers of mosquito species data, a maximum of 1000 data points per species were randomly selected for training the model. We used a radial basis function (RBF) kernel and passed the following hyper-parameters as arguments to the constructor of the support vector machine: gamma = 0.0005, C = 1.0, tolerance = 1e-10, probability = true.

The support vector machine calculated the hyperplane for the separation of both classes. With the returned binary values for the training data (positive or negative), the confusion matrix was

calculated. By re-training the parameters on a sigmoid function, the original outputs were mapped into probability values on a scale from 0 (very low probability of occurrence) to 1 (very high probability of occurrence) (Platt 1999; Pedregosa et al. 2011). This procedure is implemented by default in SCIKIT-LEARN. The latter training results were saved and used for creating distribution maps of *Ae. j. japonicus* under certain weather conditions.

Analysis of the training classes

To illustrate the different dependencies of the species on the eight weather variables, we presented the values for the two training classes ("*Ae. j. japonicus*" and "native species") at their respective sampling sites in violin plots. We additionally tested the data of both groups for significant differences ($p < 0.05$) with the Wilcoxon-Mann-Whitney test, using R and the wilcox.test-function where we set the following parameters: paired = FALSE, alternative = "two.sided".

2.3.4   Model validation

Classification

The classification result was evaluated by calculating a confusion matrix and the values of precision, recall and $f1$ score. A confusion matrix summarises the reclassification results for the set of test data (Garreta and Moncecchi 2013). Based on this matrix, the values for precision and recall are calculated for the two classes ("*Ae. j. japonicus*" and "native species"). The precision computes the proportion of true positive values (TP) from the predicted positive values, including true positive and false positive (FP) values (Eq. 2.1). By contrast, the recall calculates what proportion of the positive observations was correctly evaluated. This calculation (Eq. 2.2) considers the false negative (FN) values. The $f1$ score represents the harmonic mean of precision and recall (Eq. 2.3).

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}} \tag{2.1}$$

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}} \tag{2.2}$$

$$f1 = \frac{2(\text{PrecRec})}{\text{Prec} + \text{Rec}} \tag{2.3}$$

To calculate the training results for both classes, weighted mean values were calculated for precision, recall and $f1$ score (Eq.2.4). When x is the validation variable and n the sum of observations, then applies:

$$x = \frac{x_{japonicus}n_{japonicus} + x_{native}n_{native}}{n_{japonicus} + n_{native}} \tag{2.4}$$

Predictive power

In order to validate the predictive power of our model, we used the *Ae. j. japonicus* sampling data of 2015, which had not been included in the training, and analysed the pixel values of the predictive distribution map, referring to the weather conditions of 2015. We drew a histogram and calculated the median and interquartile range to describe the pixel values for the validation dataset.

2.3.5   Colonisation potential between 2011 and 2015

The weather conditions of individual years can differ significantly from one another and with regionally varying intensity, influencing the distribution potential of the mosquito species. We have therefore carried out a model application for the average climate values for the years 2011–2015 and produced a corresponding habitat map for *Ae. j. japonicus*.

2.4   Results

2.4.1   Analysis of the training classes

All eight weather variables show overlapping areas for both training groups, i.e. the native species and *Ae. j. japonicus* (Fig. 2.3). However, with respect to all weather variables, the distributions differ significantly from each other with $p < 0.02$.
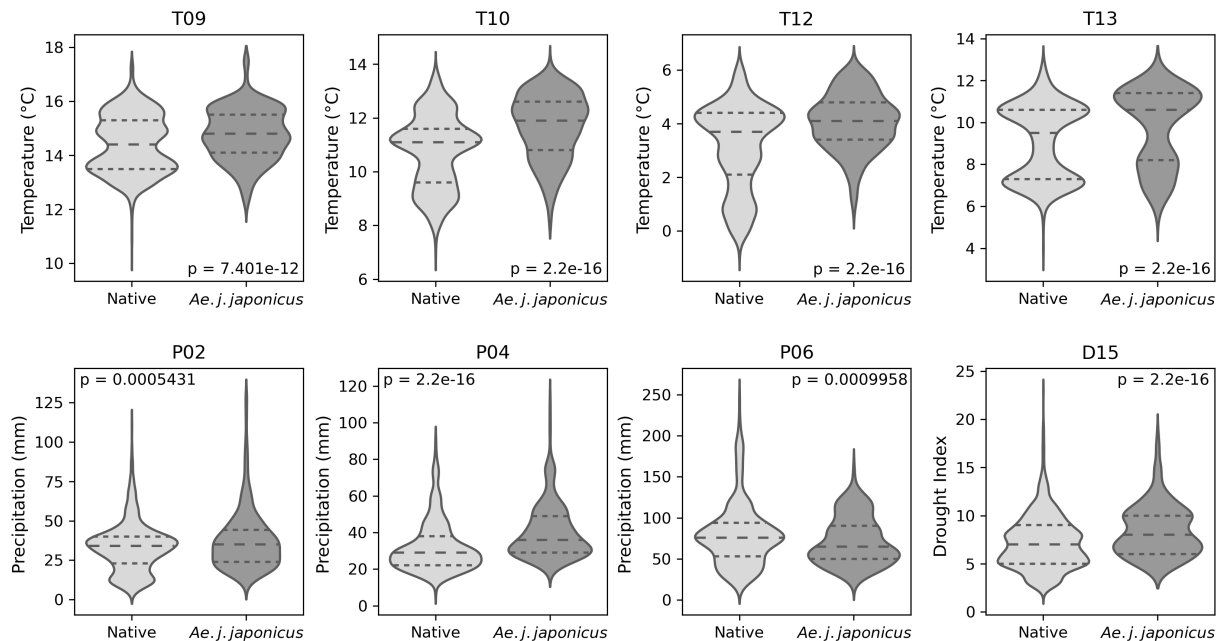
Figure 2.3: Weather conditions at the mosquito collection sites in the corresponding year of monitoring (between 2011 and 2015) classified by the training group. *Aedes vexans*, *Aedes geniculatus* and *Anopheles daciae* are grouped under the term "Native". T09 = mean temperature in September, T10 = mean temperature in October, T12 = mean temperature in December, T13 = mean temperature in spring (average of March, April and May), P02 = sum of precipitation in February, P04 = sum of precipitation in April, P06 = sum of precipitation in June, D15 = drought index of autumn (average of September, October and November). This image was created under Python 3.7

2.4.2   Model validation

Classification

The combination of our training algorithm and the chosen weather variables yielded very good classification results. As can be seen in the confusion matrix (Table 2.1), 241 of 308 test points (78.2 %) on the map, verified to be climatically suitable areas for *Ae. j. japonicus* in 2015, are correctly evaluated (true positives). Conversely, only 67 test points (21.8 %) where *Ae. j. japonicus* was present lay in the area categorised to belong to the class of native species. From the target class of native species supported by 115 test points, only 20.9 % were predicted to belong to the class of *Ae. j. japonicus*.

Regarding precision, recall and *f*1 score, the model classifies the test dataset of *Ae. j. japonicus* extremely well and only slightly less well for the test data of the native species. The *f*1 score in total (weighted average) was 79 % (Table 2.2).

Table 2.1: Confusion matrix for the trained model and validation data from 2015

		Predicted class:		Sum
		Ae. j. japonicus	Native species	
Observed class:	*Ae. j. japonicus*	241 (**TP**,TN)	67 (**FN**,FP)	308
	Native species	24 (**FP**, FN)	91 (**TN**, TP)	115
Sum		265	158	423

TP = true positive, TN = true negative, FN = false negative, FP = false positive (first place and bold referring to *Ae. j. japonicus*, in second place referring to the class of native species)

Table 2.2: Validation of the model training with test data from 2015

Class	Precision	Recall	$f1$ score
Ae. j. japonicus	0.91	0.78	0.84
Native species	0.58	0.79	0.67
Total	0.82	0.78	0.79

Predictive power

A comparison of the predicted occurrence probability for *Ae. j. japonicus* using the weather conditions and the species sampling data from 2015 indicates a high degree of accuracy of the model. This also becomes apparent in the prediction map for the year 2015 (Fig. 2.4). The occurrence probabilities for the validation points (n = 308) have a median of 0.78 and lie in the interquartile range between 0.52 and 0.81 (Fig. 2.5).

Colonisation potential between 2011 and 2015

According to the average climate conditions of the period 2011–2015 (Fig. 2.4, right), the highest colonisation potentials for *Ae. j. japonicus* (80–100 %) lie in the West German federal states of North Rhine-Westphalia (NRW), Baden-Wuerttemberg (BW), Saarland (SL) and in some parts of Rhineland-Palatinate and Hesse. In addition, there is a highly probable distribution area (60–80 %) in the southwest of Lower Saxony (LS).
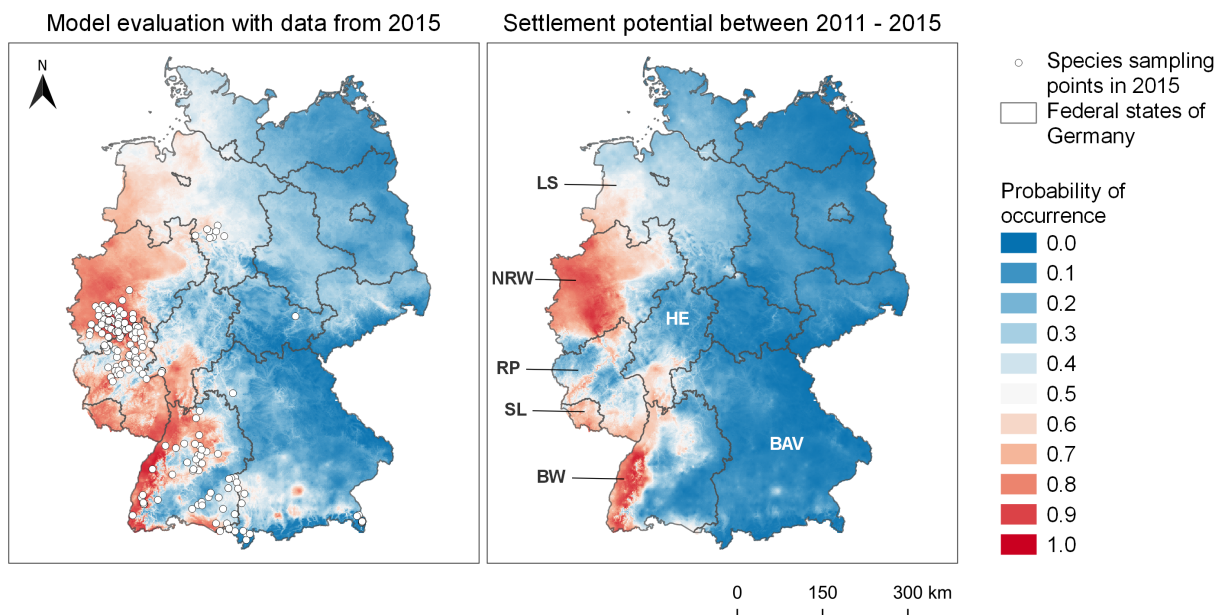
Figure 2.4: Left: predicted occurrence areas of *Ae. j. japonicus* for the year 2015 as opposed to field samplings in 2015. Species data from 2015 were not included in the model training. Right: Average colonisation potential in the period 01/2011–12/2015 (labelled federal states: LS, Lower Saxony; NRW, North Rhine-Westphalia; HE, Hesse; RP, Rhineland-Palatinate; SL, Saarland; BW, Baden-Wuerttemberg; BAV, Bavaria). This image was created with QGIS 3.8.2.
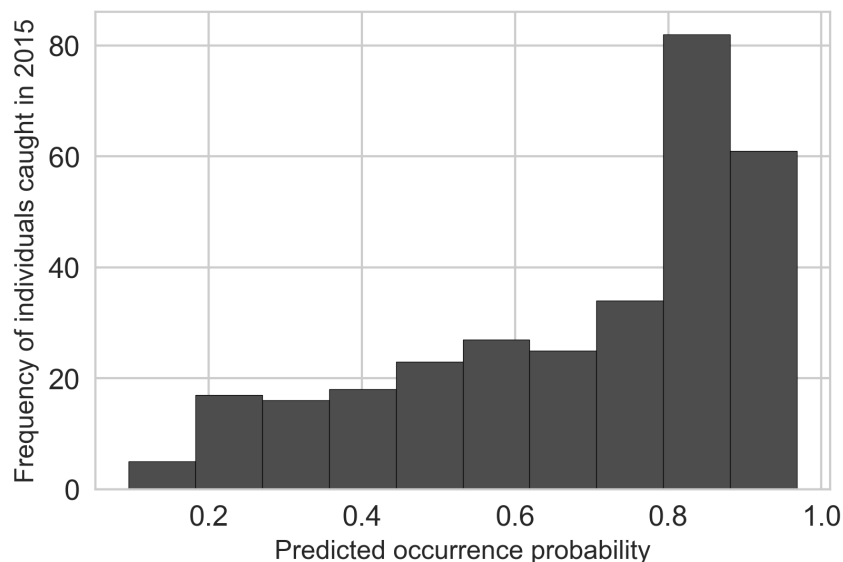
Figure 2.5: Predicted occurrence probabilities of *Aedes japonicus japonicus* in Germany, related to weather conditions of 2015, compared with field collections in 2015. This image was created with Python 2.7

2.5   Discussion

The comparison of the prediction map for the year 2015 with field collection data from 2015 (which have not been included in the model training) showed an extremely good correspondence. The concentration of predicted suitable regions for *Ae. j. japonicus* in western Germany (Baden-Wuerttemberg, Rhineland-Palatinate, Hesse, North Rhine-Westphalia) is not surprising, as we had a high amount of training data from these regions. However, the high occurrence probabilities in Bavaria (south-eastern Germany) under the weather conditions of the year 2015 is remarkable, as is the predicted spread far into the most northern regions of Germany. Indeed, *Ae. j. japonicus* specimens were not only found in 2015 in an area of roughly $900\,\text{km}^2$ in south-eastern Bavaria on the border to Austria (Zielke et al. 2016), but this species was actually even more widespread in 2016 and 2017 in large areas of southern Bavaria (Kampen et al. 2017; Koban et al. 2019).

The model application to the average climate conditions of the period 2011–2015 shows where good colonisation conditions had been since an early invasion stage during the entire period. We refer to this short period because *Ae. j. japonicus* was discovered in southern Germany (Baden-Wuerttemberg) only in 2008 and in western and northern Germany in 2012. However, in order to make predictions for a mean climate based on a longer period, the model has to be re-calibrated. With the result of long-term averaged climate conditions, it is possible to predict future developments of *Ae. j. japonicus* in Germany. The calibration and forecast for the future period of 2021–2050 are presented by Kerkow et al. (2019).

According to the map for the average climate conditions from 2011 to 2015, the climate in north-eastern Germany was less suitable than in 2015 alone. The suitable area in Lower Saxony is clearly smaller and the prediction values were low (around 10–50 %) where the population was located. This is consistent with the observation that the population seems to have decreased over the period 2011–2014, but has expanded again since 2015 (Koban et al. 2019).

In order to better assess the effectiveness of our modelling technique, we compared our results for the averaged climate conditions with those of two other models for Germany produced in completely different ways: *(i)* Melaun et al. (2015) used occurrence and weather data for 2011–2013 and seven different weather variables to train a MaxEnt model using simulated background data. The result is mapped to the climate of the period 1950–2000 and differs only slightly from ours. A completely different approach *(ii)* was carried out by Wieser et al. (2019). Using experimental life history data obtained from *Ae. j. japonicus* specimens from southern Germany (temperature-dependent development, mortality, reproduction and density-dependent larval mortality rates), the authors modelled population dynamics for two specific locations and determined where the

species can establish stable populations in Germany. The analysis, based on climate data from 1993 to 2013, reveals that the range may be larger than we have modelled. In detail, the habitats in southwestern and western Germany have a similar pattern, but the size of the suitable area in northern Germany is much larger, reaching to the far north and eastwards as far as Berlin.

This leads us to conclude that our model may underestimate the possible spread of *Ae. j. japonicus* in Germany. Although we have already used occurrence data of four years for the training, they may not yet be sufficient for all suitable climatic conditions. However, our result is remarkably congruent with that of the MaxEnt model and thus able to compete with a well-established and widely used method.

A possible problem with data-driven species distribution models, apart from the possibility that not all environmental conditions are reflected, is the quality of the training data. In our case, 70 % of our data originate from the citizen science project "Mückenatlas". Thus, the modelling results may be slightly biased, as the majority of mosquitoes submitted by the participants are most likely from densely populated regions. However, about 30 % of the training data originate from systematic field collections which particularly concentrated on the margins and surroundings of known distribution areas of *Ae. j. japonicus* (Kampen et al. 2016a).

Regarding the selection of mosquito species to replace absence data in machine learning, the question arises as to why we chose the species *Ae. vexans*, *Ae. geniculatus* and *An. daciae*. Our modelling approach is driven solely by climate data, and the aim was to determine the climatic-ecological niche of the Asian bush mosquito. We therefore searched for mosquito species, which, when considering some relevant climate parameters (mean annual and summer precipitation, drought indices and temperature data), seemed to differ in their occurrence spectrum from that of *Ae. j. japonicus*. We have tested different species combinations in the initial phase of modelling and found, firstly, that the use of more or less than three species tends to diminish the results measured by the total $f1$ score. Secondly, the combination of the two common species *Ae. vexans* and *Ae. geniculatus* together with the species *An. daciae*, which presumably prefers more continental conditions, achieved particularly good results. In a later modelling phase, we optimised the selection of climate variables by means of a deep learning method (Wieland et al. 2017). The existence of competitive conditions was not a criterion for the selection of the species. Competitive situations for mosquitoes result from oviposition and development of pre-imaginal stages in the same habitats. Amongst our species selected for modelling, only the dendrolimnobiotic species *Ae. geniculatus* (Becker et al. 2011) occasionally lays its eggs in the same water sources as *Ae. j. japonicus* (Damiens et al. 2014; Seidel et al. 2016).

Another interesting aspect of our result is that our model performs very well in validation, although the weather and climate data of the two training classes "native species" and "*Ae. j. japonicus*" are noisy and partly overlap (Fig. 2.4). Consequently, the support vector machine was able to handle this circumstance effectively. This is probably due to the use of the RBF kernel, which can identify complicated distribution patterns in the data. When comparing different algorithms and their combinations for our study (Früh et al. 2018), this algorithm also achieved the best result of all individually applied algorithms. However, if more mosquito data become available in the future via the citizen science project, we would consider using learning algorithms that are much more powerful in general, such as XG-Boost (Chen and Guestrin 2016; Brownlee 2017). The main strength of the support vector machine lies in the handling of smaller amounts of data (Pedregosa et al. 2011; Nalepa and Kawulok 2019).

As mentioned before, mesoclimate data with a resolution of $1\,km^2$ can only give an approximate picture of the suitable habitats and thus a possible distribution of a mosquito species. At the local level, landscape forms and the resulting micro-habitats also play a role. It is also possible that unfavourable climatic conditions may be compensated in some way. For example, in regions with low precipitation, people may regularly provide water-filled breeding habitats such as rain water barrels or flowerpots. Therefore, in a further step, the results of this model were combined with landscape and wind data, which led to a significant improvement of the results as measured by the hit rate for *Ae. j. japonicus* (Kerkow et al. 2019).

2.6   Conclusions

Our model approach appears to be suitable for predicting the distribution area of the Asian bush mosquito *Ae. j. japonicus* in newly invaded areas. The results were achieved by the exclusive use of presence data of this species and three native species. The model output matches extremely well with presence data from 2015, which were not included in the model training. Due to the use of presence-only data, the method presented here is well-suited to datasets that are based on passive monitoring programmes such as citizen science projects. Our result is consistent with that of another data-driven machine-learning approach that used generated absence data (Melaun et al. 2015). However, when applying the method, it is important to bear in mind that the dispersal potential of an invasive species is underestimated if the dataset is too small to represent the realised ecological niche of a species.

With the help of large-scale, long-term averaged climate data, only a rough impression of the possible further spread of invasive mosquito species in Germany can be obtained, independent

of the modelling technique. On a smaller scale, land use forms correlating with the presence of certain breeding habitats and causing certain microclimates have an additional important impact.

Data availability   Mosquito occurrence data used in this model are provided in the supplementary information files. We uploaded four Excel tables, one for each mosquito species, containing the year of discovery, the geo-coordinates and the weather values relevant for both model training and validation. The CULBASE database is not yet publicly accessible, further mosquito data are available on reasonable request to DW or HK.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no conflict of interest.

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Chapter 3

Nested modelling approach based on fuzzy set theory

What makes the Asian bush mosquito *Aedes japonicus japonicus* feel comfortable in Germany? A fuzzy modelling approach

Authors   Antje Kerkow[a,b], Ralf Wieland[a], Marcel B. Koban[a], Franz Hölker[b,c], Jonathan M. Jeschke[b,c,d], Doreen Werner[a], Helge Kampen[e]

a   Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany
b   Freie Universität Berlin, Department of Biology, Chemistry, Pharmacy, Institute of Biology, Königin-Luise-Str. 1-3, 14195 Berlin, Germany
c   Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany
d   Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstr. 34, 14195 Berlin, Germany
e   Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald - Insel Riems, Germany

3.1   Abstract

Background   The Asian bush mosquito *Aedes japonicus japonicus* is an invasive species native to East Asia and has become established in North America and Europe. On both continents, the species has spread over wide areas. Since it is a potential vector of human and livestock pathogens, distribution and dissemination maps are urgently needed to implement targeted surveillance and control in case of disease outbreaks. Previous distribution models for Europe and Germany in particular focused on climate data. Until now, effects of other environmental variables such as land use and wind remained unconsidered.

Results   In order to better explain the distribution pattern of *Ae. j. japonicus* in Germany at a regional level, we have developed a nested approach that allows for the combination of data derived from *(i)* a climate model based on a machine-learning approach; *(ii)* a landscape model developed by means of ecological expert knowledge; and *(iii)* wind speed data. The approach is based on the fuzzy modelling technique that enables to precisely define the interactions between the three factors and additionally considers uncertainties with regard to the acceptance of certain environmental conditions. The model combines different spatial resolutions of data for Germany and achieves a much higher degree of accuracy than previous published distribution models. Our results reveal that a well-suited landscape structure can even facilitate the occurrence of *Ae. j. japonicus* in a climatically unsuitable region. Vice versa, unsuitable land use types such as agricultural landscapes and coniferous forests reduce the occurrence probability in climatically suitable regions.

Conclusions   The approach has significantly improved existing distribution models of *Ae. j. japonicus* for the area of Germany. We generated distribution maps with a resolution of $100\,\text{m} \times 100\,\text{m}$ that can serve as a basis for the design of control measures. All model input data and scripts are open source and freely available, so that the model can easily be applied to other countries or, more generally, to other species.

3.2   Background

The Asian bush mosquito *Aedes japonicus japonicus* (Theobald, 1901) (Diptera: Culicidae), native to Japan, Korea, Taiwan, China and south-eastern Russia [1], is an invasive species of currently great importance in the northern hemisphere, especially within temperate climatic areas,

although it has also been discovered in subtropical and tropical regions such as Florida and Hawaii [2, 3, 4, 5, 6]. The first record of the species in Europe was in France in 2000. Since then, it has been detected in 12 European countries [7, 8, 9, 10]. In Germany, the species was discovered in 2008 at the Swiss border [11]. Meanwhile, *Ae. j. japonicus* is widespread in the country. Its main areas of distribution are in the west, southwest and southeast [8].

Although it has been assumed that it will be no longer possible to eliminate *Ae. j. japonicus* completely from Germany by means of control measures [8], its continuing spread is closely monitored because the species is a potential vector of disease agents of humans and livestock. Its vector competence includes at least 11 different viruses (including West Nile virus and Zika virus) and two filarial nematode species [12, 13, 14].

The aim of this study is to use modelling and the integration of ecological expert knowledge to generate maps that show the risk of colonisation with *Ae. j. japonicus* in Germany as accurately and detailed as possible. The maps should *(i)* be suitable for informing the public to support preventive measures and initiate targeted control measures in the event of a disease outbreak for whose agent *Ae. j. japonicus* is a competent vector and *(ii)* assess how the species will spread under the influence of climate change.

Distribution models for *Ae. j. japonicus* already exist for Europe [15] and, more specifically, for Germany [16, 17] and Slovenia [7]. They rely on climate data such as precipitation sums and average monthly and seasonal temperatures with a resolution of 1 km × 1 km to 10 km × 10 km, and partly on elevation data [7]. As these distribution models score well in their validation, it can be concluded that climatic factors are relevant for the species to become established in a region. However, the distribution maps derived from climate models are not suitable for planning concrete control measures due to their usually broad scale. It should also be noted that recently introduced species may not yet be in equilibrium with their environment, so that the ecological niche determined by machine learning is often calculated too narrowly [15]. In addition, the occurrence of mosquitoes is strongly dependent on local weather events, which can sometimes deviate dramatically from average climatic conditions.

On a smaller scale, the occurrence of certain mosquito species can be predicted using landscape data (see for example [18, 19, 20]). Landscape data are an indicator of the occurrence of breeding sites for which each mosquito species has its own requirements. Egg deposition and larval development of the Asian bush mosquito take place in small containers, both in natural habitats, such as stream rock pools and tree holes of deciduous trees [1], and in artificial containers like plant dishes, rainwater catchments and trash cans, the latter particularly often being available in human settlements [11, 21, 22, 23]. The landscape does not only affect egg deposition and

larval development. It can, for instance, also be correlated with the occurrence of blood hosts and predators. Besides climate and landscape (including land use and further landscape elements), we found that regional mean wind speeds also seem to have an important influence on the presence of *Ae. j. japonicus*.

Based on the assumption that the three factors "climate", "landscape" and "wind" mainly determine the possible distribution of the species, the question arises with which model type the interaction of the corresponding geodata can be expressed. Requirements for implementation were that *(i)* it is comprehensible to biologists; *(ii)* it allows uncertainties; and *(iii)* the interplay of the factors can be controlled by the modeller. The first point is important as ecological knowledge about *Ae. j. japonicus* is incomplete. As soon as new results from ecological studies emerge, the model can be adapted. The second requirement results from the fact that biological expert knowledge is often expressed by use of linguistic terms instead of exact numbers, which in turn is mainly due to the fact that individuals within a species show a variability with regard to the acceptance of certain environmental conditions.

The fuzzy modelling technique meets all the mentioned criteria. It is a white box modelling approach that allows for the integration of biological expert knowledge [24] and enables the influence of each input variable on the model to be tracked and easily understood by biologists without an informatics background. The fuzzy approach [25] can deal with uncertainties and is ideal for habitat models, as its basic idea is that assignments do not always have to follow Boolean principles, but that there is often a degree of membership. For a habitat model, each environmental variable that is relevant to the species can be divided into fuzzy sets, which are given a name, a so-called linguistic term. For example, if the environmental variable is "wind speed", it could be divided into the fuzzy sets "comfortable" (unrestricted flight capability), "high" (causing moderate flight restrictions) and "too high" (causing strong flight restrictions). By means of membership functions, values are assigned to the sets with every value having degrees of membership to the sets on a percentage scale. The interplay of environmental variables and their different states can be directly controlled by the modeller with the help of rules.

There are already numerous studies on the ecology of the Asian bush mosquito available, and fuzzy modelling is an established method in ecological niche modelling (see e.g. [26, 27, 28]). Our particular research questions were therefore *(i)* whether fuzzy modelling allows to combine models developed on the basis of machine learning (a climate model), expert knowledge (a landscape model) and additional important data (wind) in such a way that more accurate predictions can be achieved compared to the initial models (landscape only and climate only), and *(ii)* whether, despite the originally different resolutions of the input data ($100\,\text{m} \times 100\,\text{m}$, $200\,\text{m} \times 200\,\text{m}$ and

$1000\,\text{m} \times 1000\,\text{m}$), the outcome, calculated for the finest scale, performs better in the validation than that of the most detailed input model (landscape). As the novelty of the approach is the combination of local landscape and wind data with large scale mean climate data by means of fuzzy logic, as well as the interplay of ecological expert knowledge and the power of machine learning, we call the approach hereafter a nested approach.

3.3 Methods

3.3.1 Habitat requirements and selection of model input parameters

For the selection of input data of the fuzzy model, and especially for the development of the landscape model on which the fuzzy model is partly based, both ecological characteristics of the species as well as generally favourable conditions for the occurrence of mosquitoes were considered. To improve our understanding of the species and its potential habitats in the study area we reviewed the literature, talked to other mosquito specialists and statistically assessed various geodata and satellite images of Germany. A summary of the habitat requirements is presented here in order to understand the setup of the model.

Habitat choice of mosquitoes is basically driven by the availability of suitable breeding sites for egg deposition and larval development. *Ae. j. japonicus* uses small breeding habitats and naturally occurs in stream rock pools, kinked bamboo trunks and tree holes of deciduous trees [1]. It can also be found in human settlements, where the larvae develop in small artificial containers, including plant dishes, buckets, trash cans, discarded snack bags, rainwater catchments, fountains and used tires [11, 21, 22, 23]. Shade is also beneficial for both larvae and adults of the species as it minimises the risk of breeding site evaporation and desiccation and provides resting places during hot days [2, 22, 29]. The general availability of plants, flowers and fruits is important for mosquito adults, as they feed on plant juices and nectar. Organic material such as leaf litter and pollen is equally important for the larvae, as they feed on detritus and bacteria [22, 30, 31]. For egg production, female mosquitoes need proteinaceous blood meals. *Ae. j. japonicus* females were observed to feed on mammals (such as white-tailed deer, fallow deer, horses and humans) and birds, but not on amphibians or reptilians [11, 32, 33].

Regarding the parameter terrain height, we find a negative correlation of *Ae. j. japonicus* occurrence with height by intersecting collection data with an elevation map ($25\,\text{m} \times 25\,\text{m}$ resolution) and by considering small areas (about $10\,\text{km} \times 10\,\text{km}$), which confirms findings of a study in Japan [29]. It seems that *Ae. j. japonicus* prefers valleys over higher altitudes. When

looking at the area of Germany, however, there is no relationship between elevation and the occurrence of the mosquito. As land use and climate, which sometimes correlate with height, did not explain the observed distribution pattern, we suspect that the correlations for smaller areas are rather due to wind speed.

To our knowledge, no study exists about how the behaviour and distribution of *Ae. j. japonicus* are affected by wind. However, the flight activity of haematophagous insects can be greatly influenced by wind, and females of most mosquito species drastically reduce host-seeking flights when wind speeds are greater than about 3 km/h (0.83 m/s) [34]. Some mosquito species have been observed to fly close to the ground and to cling to the vegetation above certain wind speeds, e.g. *Aedes albopictus* [35]. In fact, wind speed affecting the mosquito flight behaviour is known to be species-specific (the wind speed threshold at which mosquitoes stop flying was reported to be between 3 km/h (0.83 m/s) for species in central Alaska and 29 km/h (8.06 m/s) for Canadian sub-arctic species [34]), and thus could serve as an indicator to describe the ecological niche of a species.

3.3.2   Data

Species distribution data

Species collection data were relevant for the model to analyse and select environmental input data (to complete our understanding of the ecological dependencies) as well as to evaluate the model. They were derived from the German mosquito database "CULBASE" [36], which contains data from active and passive mosquito monitoring approaches. The passive monitoring data originate from the citizen science project "Mückenatlas" [37] and the active monitoring data from inspections of regions and their adjacent areas from which invasive mosquito species were submitted. In the latter case, possible breeding habitats were screened for larvae, and traps were set up in some cases [37]. At the time of download (10 April 2018), the database included 1110 records of *Ae. j. japonicus* sampling sites from 2012–2017, 79 % of them linked to passive monitoring. The distribution of the species in Germany regarding to this update is shown in Fig. 3.1.

Model input data and transformations

The fuzzy model is based on three submodels that process appropriate geodata (climate data, land use data and wind data). To integrate all datasets into the model, they were pre-processed in several steps and harmonised in terms of file type, coordinate system, grid cell resolution and
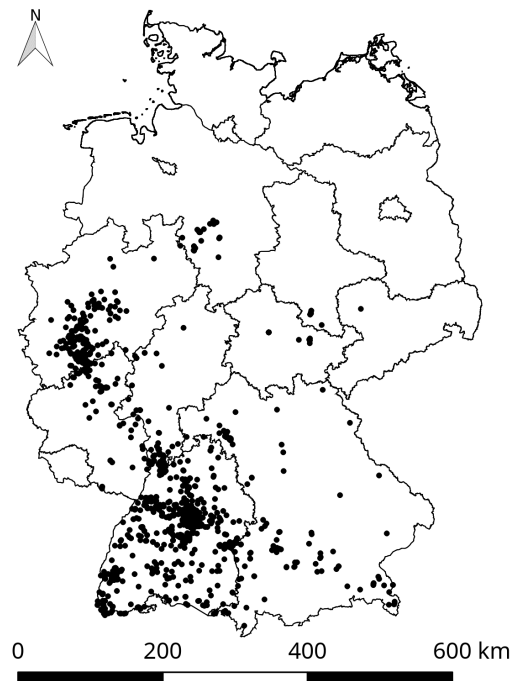
Figure 3.1: Collection sites of *Aedes japonicus japonicus* in Germany. The collection sites (n = 1110) are indicated as black dots and include samplings from the years 2012–2017. The geodata of Germany originate from the Bundesamt für Kartografie und Geodäsie [58]

raster alignment (Fig. 3.2). Finally, the model input data were saved as grid files with a resolution of 100 m × 100 m in the coordinate system DHDN Gauss-Kruger-Zone 3 (EPSG 31467). Data processing was done with the GDAL (1.11.3) library and Python (2.7). Additionally, the GRASS GIS tool "r.resample" was used to calculate the grid orientations.

Climate data

A dataset based on the approach by Kerkow et al. (2020)[1] and Früh et al. (2018) [17] was included into the fuzzy model. It defines the climate suitability of *Ae. j. japonicus* as values between zero and one, within Germany depending on climate variables according to Wieland et al. (2017) [38]. The underlying data have a resolution of 1 km × 1 km and are derived from the German Weather Service [39]. They include the monthly precipitation sums of February, April and June, the autumn (average of September, October and November) drought index, the average monthly temperatures of September, October and December, and the average seasonal temperature of spring (average of March, April and May). The model is based on a support vector machine

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[1]This reference is not included in the published version of this article.
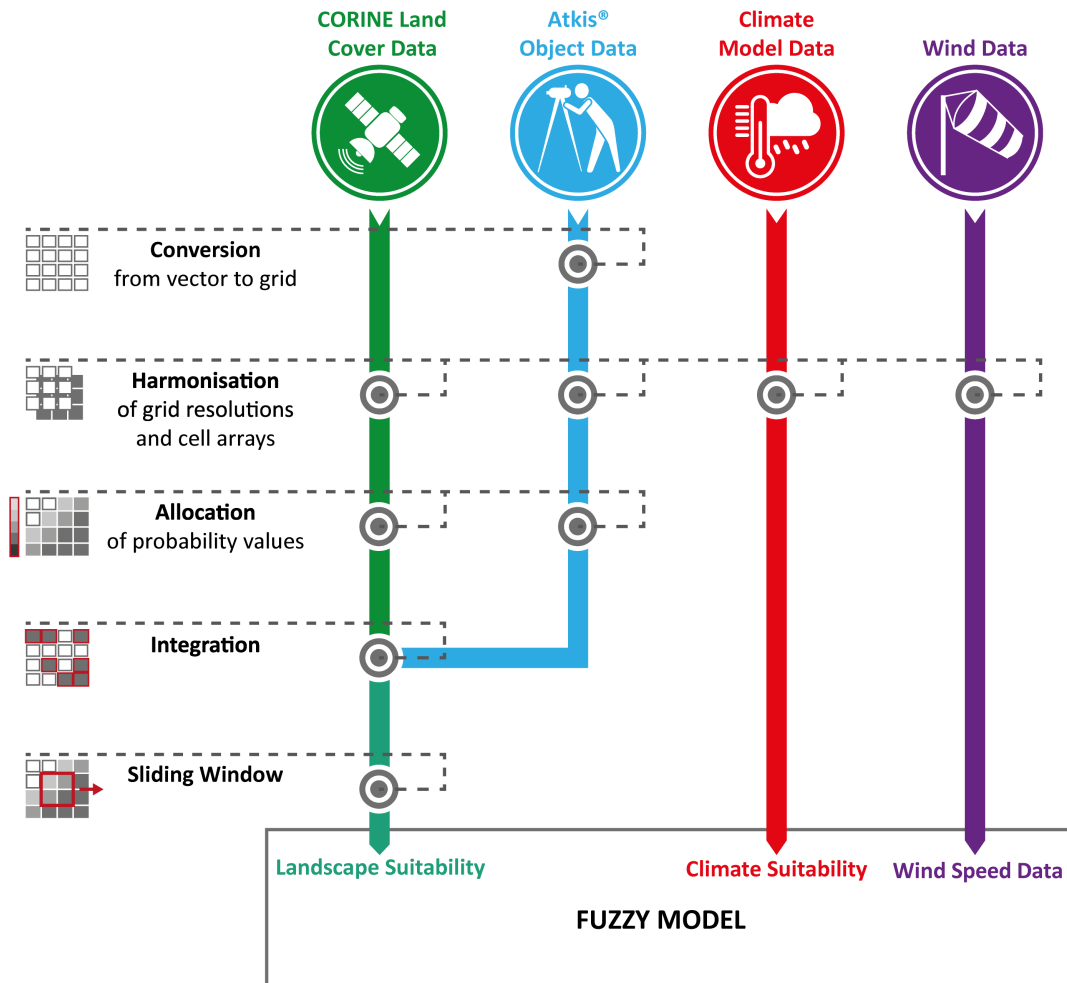
Figure 3.2: Input data of the fuzzy model and steps of pre-processing

[40] as a training algorithm that distinguishes the climate niche of *Ae. j. japonicus* from the niche of three mosquito species native to Germany (*Aedes vexans*, *Aedes geniculatus* and *Anopheles daciae*).

To transform the climate model trained for the period 2012–2014 into a long-term climate model, a calibration was performed with mean climate data for the period of 1981–2010 (most recent international climate reference period) and an updated set of field collection data from 2012–2017. Ten percent of the collection data was reserved for k-fold cross validation. Additionally, we changed the data scaling before starting the training and calculated it by Eq. 3.1, with x being the input and s(x) being the scale(x):

$$s(x) = \frac{x - \bar{x}}{\sigma^2}; \bar{x} = mean(x); \sigma^2 = variance(x) \tag{3.1}$$

To forecast the future distribution of *Ae. j. japonicus* until 2050, we changed the input variables of the climate model according to the average of several IPCC climate change scenarios from the ATEAM project (HadCM3 SA1, HadCM3 SA2, HadCM3 SB1, HadCM3 SB2, CGCM2 SA2, CSIRO2 SA2 and PCM SA2) [41] and reapplied it. On average, summer temperature increased by 1.4 K, summer precipitation declined by 4 %, and winter precipitation increased by 5 %.

Land use data

Land use data were derived from satellite image interpretations from the CORINE Land Cover database [42] and the ATKIS vector data from the State Survey Authority [43]. The satellite image interpretations (Europe-wide dataset) had a resolution of 100 m × 100 m and included 44 different land use types. From the ATKIS data, we extracted additional datasets that were relevant for the occurrence of the species. The vector data were gridded by assigning a 100 m × 100 m grid cell from 51 % fill level onwards. Considering the habitat requirements of the species, we assigned suitability values between zero (no suitability) and one (very good suitability) for each type of land use (Tables 3.1, 3.2).

Landscapes were classified as completely unsuitable if they either did not meet the known habitat requirements, as is the case with non-irrigated arable land and sparsely vegetated areas (because of their lack of shade and breeding sites), or with large open waters (which do not serve as breeding sites inter alia due to the presence of predators), or if they simply have not been reported to be appropriate habitats for *Ae. j. japonicus*. The latter applies to moors and heathland, beaches and dunes, glaciers and places with perpetual snow, marshes, and peat bogs.

Land use types and landscape structures that we assumed to be particularly suitable and that have been reported to be hot spot occurrence areas included: *(i)* broad-leaved and mixed forests (due to the availability of shade and resting sites); *(ii)* green urban areas; *(iii)* sport and leisure facilities; *(iv)* harbours (the last three mentioned due to their diverse habitat structure and the availability of breeding sites in the form of trash); *(v)* cemeteries (both due to the flower vase density and the abundance of flowers whose nectar serves as food, and because of its structural diversity including shady resting sites [44]); *(vi)* gardens (due to the availability of small water-filled containers such as rain barrels and flower pots and a similar landscape structure as cemeteries); *(vii)* zoological gardens (due to the high abundance of blood-feeding hosts, animal drinking sta-

Table 3.1: CORINE land use data. Suitability of land use types for the occurrence of *Aedes japonicus japonicus* with the attributes being derived from the CORINE Land Cover dataset

No.	CORINE land use category	Degree of suitability (from 0 to 1)	Percentual area of Germany	No.	CORINE land use category	Degree of suitability (from 0) to 1)	Percentual area of Germany
1	Continuous urban fabric	0.2	0.04	23	Broad-leaved forest	0.9	9.73
2	Discontinuous urban fabric	1.0	6.91	24	Coniferous forest	0.1	16.57
3	Industrial or commercial units	0.2	1.38	25	Mixed forest	0.8	4.08
						0.8	4.08
4	Road and rail networks and associated land	0.5	0.06	26	Natural grasslands	0.0	0.42
5	Port areas	0.8	0.02	27	Moors and heathland	0.0	0.27
6	Airports	0.2	0.11	28	Sclerophyllous vegetation	–	–
7	Mineral extraction sites	0.0	0.20	29	Transitional woodland-shrub	0.6	0.63
8	Dump sites	0.2	0.04	30	Beaches, dunes, sands	0.0	0.03
9	Construction sites	0.3	0.01	31	Bare rocks	0.1	0.04
10	Green urban areas	1.0	0.20	32	Sparsely vegetated areas	0.0	0.03
11	Sport and leisure facilities	1.0	0.45	33	Burnt areas	–	–
12	Non-irrigated arable land	0.0	37.92	34	Glaciers and perpetual snow	0.0	3.63
13	Permanently irrigated land	–	–	35	Inland marshes	0.0	0.09
14	Rice fields	–	–	36	Peat bogs	0.0	0.21
15	Vineyards	0.1	0.35	37	Salt marshes	0.0	0.05
16	Fruit trees and berry plantations	0.3	0.42	38	Salines	–	–
						–	–
17	Olive groves	–	–	39	Intertidal flats	0.0	0.05
18	Pastures	0.2	17.98	40	Water courses	0.0	0.21
19	Annual crops associated with permanent crops	–	–	41	Water bodies	0.0	0.89
20	Complex cultivation patterns	0.1	0.20	42	Coastal lagoons	0.0	0.05
21	Land principally occupied by agriculture, with significant areas of natural vegetation	0.2	0.27	43	Estuaries	0.0	0.03
22	Agro-forestry areas	–	–	44	Sea and ocean	0.0	0.06

Table 3.2: ATKIS land use data. Suitability of land use types for the occurrence of *Aedes japonicus japonicus* with the attributes being derived from the ATKIS dataset

ATKIS object category	Degree of suitability (from 0 to 1)	Percentual area of Germany
Cemeteries	1.0	0.11
Landfill sites	0.2	0.05
Garden centres	1.0	0.05
Gardens	1.0	0.37
Zoological gardens	1	0.01

tions that could function as breeding sites, and their diverse park-like landscapes [45]); and *(viii)* garden centres (where plenty of nectar and water-filled flower pots are available).

The CORINE and ATKIS suitability arrays were combined, overwriting the CORINE data with the higher-resolution, selected ATKIS data when available. We considered the interactions of neighbouring landscape elements by applying the sliding (or moving) window technology [46, 47]. The sliding window calculates the mean value for each grid cell and its surrounding cells within a certain distance. This leads to the result that highly rated cells in the neighbourhood of poorly rated cells become less highly rated and cells with originally low suitability can get upgraded by a very suitable neighbourhood. We tested sliding windows with sizes of $100\,\text{m} \times 100\,\text{m}$ to $1100\,\text{m} \times 1100\,\text{m}$ ($100\,\text{m}$ corresponds to one raster cell) at a stepwise enlargement by $200\,\text{m}$ edge length each, because the window required an uneven pixel number. Then we intersected the outcome with the *Ae. j. japonicus* occurrence data and chose the window with $700\,\text{m}$ edge length, which best described the natural distribution of the species, supposing that a successive increase of findings should be given with increasing degrees of landscape suitability. The resulting array, containing the suitability values from zero to one, was used as model input.

Wind data

Wind data were provided by the Climate Data Centre of the German Weather Service [39] and downloaded for the most recent international climate reference period of 1981–2010. They have a resolution of $200\,\text{m} \times 200\,\text{m}$ and rely on a statistical wind field model, which considers measurements $10\,\text{m}$ above the ground as well as the geographical location, terrain and type of land use. The data are represented by continuous real values.

Fuzzy rule-based modelling

For each input dataset (climate suitability, wind speed and landscape suitability), we defined fuzzy sets by giving membership functions to linguistic terms, e.g. "wind speed is comfortable", "wind speed is high" or "wind speed is too high" (Fig. 3.3). The membership functions were derived from statistical analyses with the *Ae. j. japonicus* occurrence data and the input raster maps of the model. We set nine thresholds to define occurrence probability values for the model output (Fig. 3.4). The value "bbbb" represents the lowest occurrence probability ("b" for "bad"), "m" a medium occurrence probability and "gggg" the highest occurrence probability ("g" for "good"). In the next step, we defined the fuzzy rules (Table 3.3).
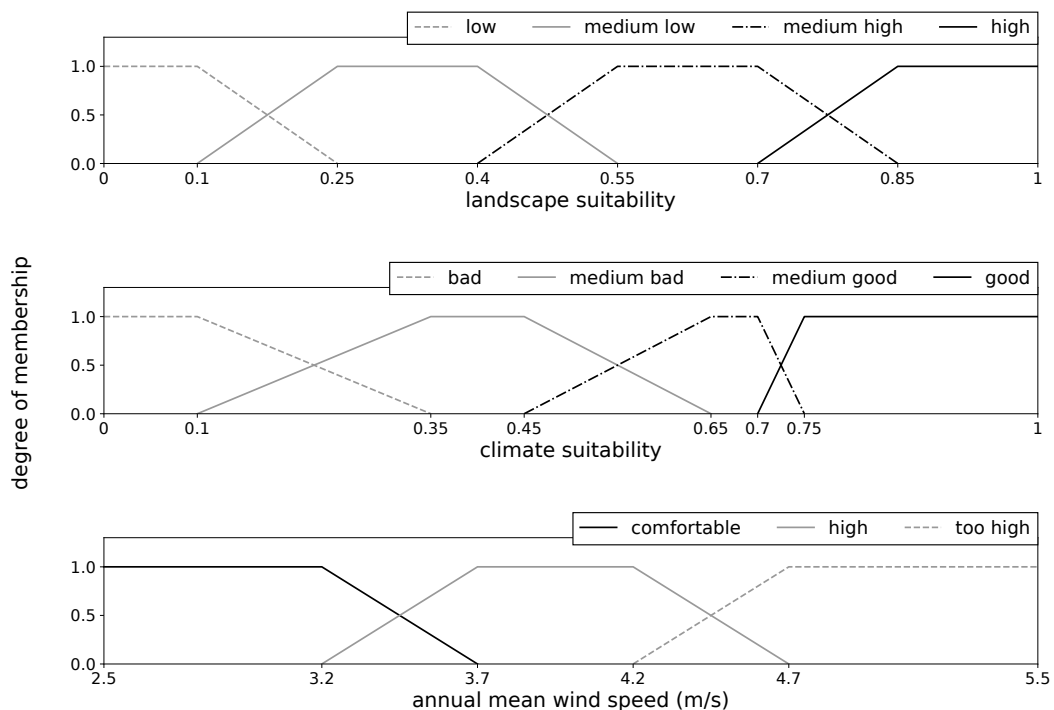
Figure 3.3: Membership functions of the fuzzy model

Software and implementation

The tool Samt2Fuzzy from the software SAMT2 [48, 49] was used for implementing the fuzzy model. After applying the model, an output raster was created and saved as grid file using Python 2.7. The calculation time for one model application was 20.25 min on a computer with an Intel Xeon CPU E5-1620 v2 (3.70 GHz) processor under Ubuntu 16.04 (xenial). Detailed maps were generated with QGIS 2.14.
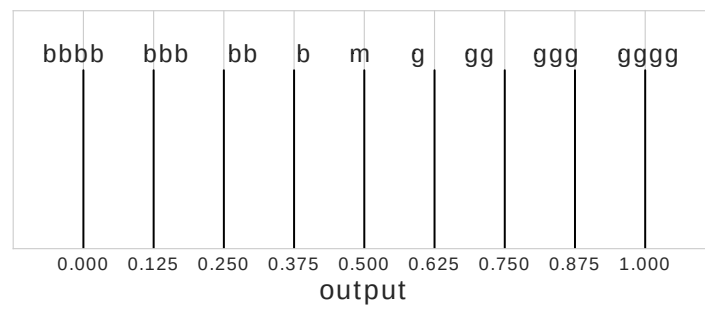
Figure 3.4: Output definitions of the fuzzy model

3.4 Results

3.4.1 Results of input models

Of the three input variables of the model (Fig. 3.5), two were based on submodels: landscape suitability and climate suitability. The climate model calculated for the actual weather conditions (1981–2010) reached an accuracy of 84.13 % under 40-fold repeated trainings with a standard deviation of 1.22 %. The intersections of the climate map with the occurrence points of *Ae. j. japonicus* yielded a median prediction value of 0.78 (mean 0.68).

landscape suitability	wind speed (m/s)	climate suitability
0.00	≤ 2.2	0.00
0.25	2.9	0.25
0.50	3.6	0.50
0.75	4.3	0.75
1.00	≥ 5.0	1.00
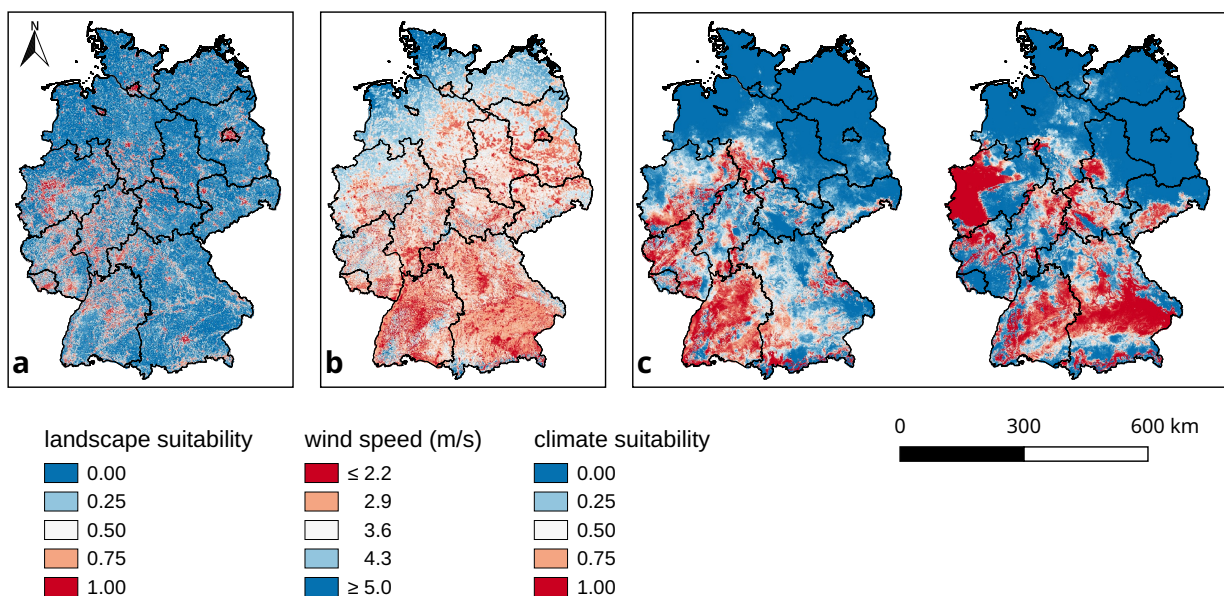
Figure 3.5: Model input data. Landscape suitability (**a**), mean wind speed in the period 1981–2010 (**b**), and climate suitability for the periods 1981–2010 (left) and 2021–2050 (right) (**c**). The geodata of Germany originate from the Bundesamt für Kartografie und Geodäsie [58]

For the landscape suitability model, the sliding window script was applied after assigning the suitability values for each land use type. The question arose how big the window had to be. An application for the number of seven pixels (corresponding to 700 m) turned out to be the most suitable distance measure. As shown in Fig. 3.6, the land use probabilities at the observed occurrence points of *Ae. j. japonicus* in Germany changed from a bimodal distribution to a unimodal left-skewed distribution, which better reproduces the real environmental conditions. When the number of pixels was increased to nine, the curve became bell-shaped and thus inappropriate for representing the relationship of landscape suitability and the number of species samplings. Figure 3.7 shows a section of the resulting land use dataset and how it developed by applying the

sliding window technique. Intersecting the outcome of the landscape suitability model with the *Ae. j. japonicus* occurrence data gave a median prediction value of 0.75 (mean 0.71).
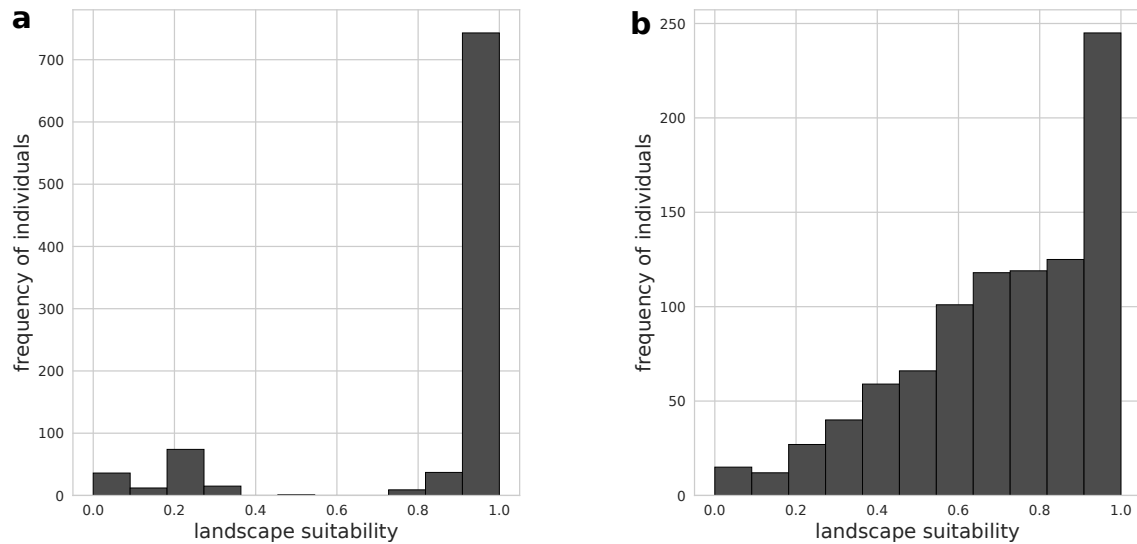
Figure 3.6: Procedure for selecting the pixel size of the sliding window. Occurrence probabilities at the collection sites of *Aedes japonicus japonicus* in Germany (1110 samplings), depending on the pre-processed land use data before applying the sliding window technique to the data (**a**) and after applying the technique with 700 m as a distance parameter (**b**)

The membership functions of the fuzzy model defined for each input dataset (landscape suitability, climate suitability and mean annual wind speed) were derived from statistics that compare the distribution of the values over the entire area of Germany with the distributions at the sites where the mosquito species occurs (Fig. 3.8). For all parameters, the distribution curves at the sites of discovery clearly differed from the distributions over the entire area of Germany. Strikingly, *Ae. j. japonicus* was not shown to occur in regions of Germany characterised by wind speeds higher than 4.7 m/s. Therefore, special attention was paid to the input variable "wind" when defining the fuzzy rules (Table 3.3). At average wind speeds of 3.7 m/s, the suitability for the occurrence of *Ae. j. japonicus* is already significantly reduced according to the model and at an average wind speed of 4.7 m/s, the model reduces habitat suitability to a maximum of 25 %. Figure 3.9 displays the consequences of the fuzzy rule definitions on a metric scale.

3.4.2 Results of the fuzzy model

Our results (Fig. 3.10) provide a very detailed picture of how the Asian bush mosquito could spread under current and future climatic conditions. The prediction maps for both actual and future conditions reveal that urban areas are generally suitable for occurrence. Under current
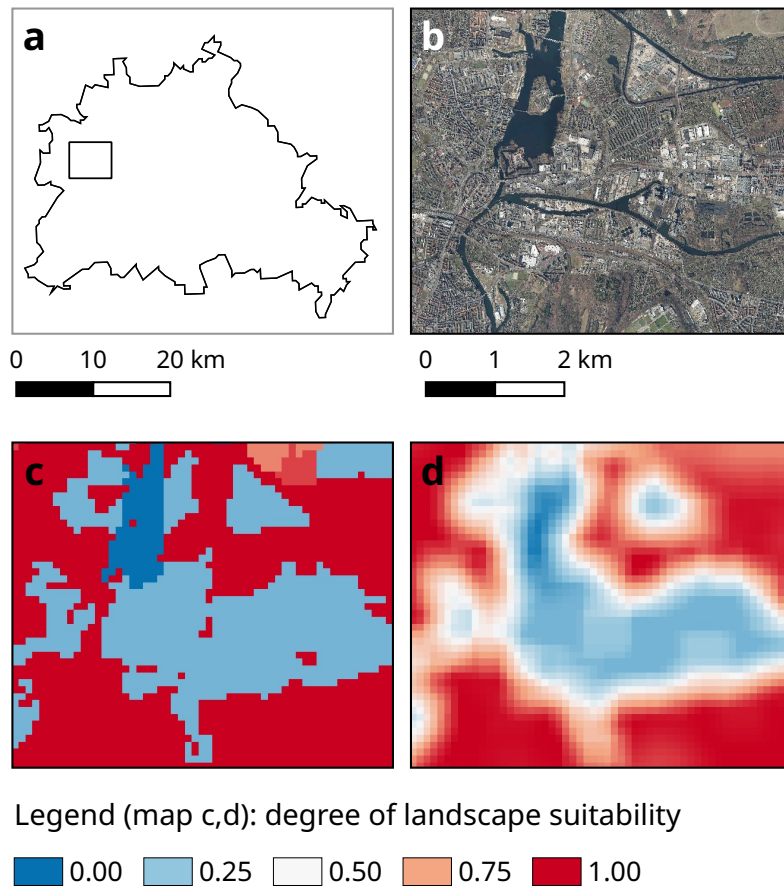
Figure 3.7: Illustration of the effect of the sliding window on the data. Image section from Germany-wide datasets of land use data in the area of Berlin. **a** Outline map. **b** Aerial photographs of the section (Senatsverwaltung für Stadtentwicklung und Wohnen Berlin, 2016). **c, d** Land use suitability maps based on the CORINE and ATKIS land use data before (**c**) and after (**d**) the application of the sliding window technique. These maps illustrate the model input factor "landscape suitability" on a large scale. A random area in the city of Berlin was chosen, where aerial photographs [59] can be freely utilised. The area mainly consists of continuous and discontinuous urban fabric, road and rail networks and associated land, green urban areas, gardens and water bodies. The outline map for the area of Berlin originates from geodata of the Bundesamt für Kartografie und Geodäsie [58]
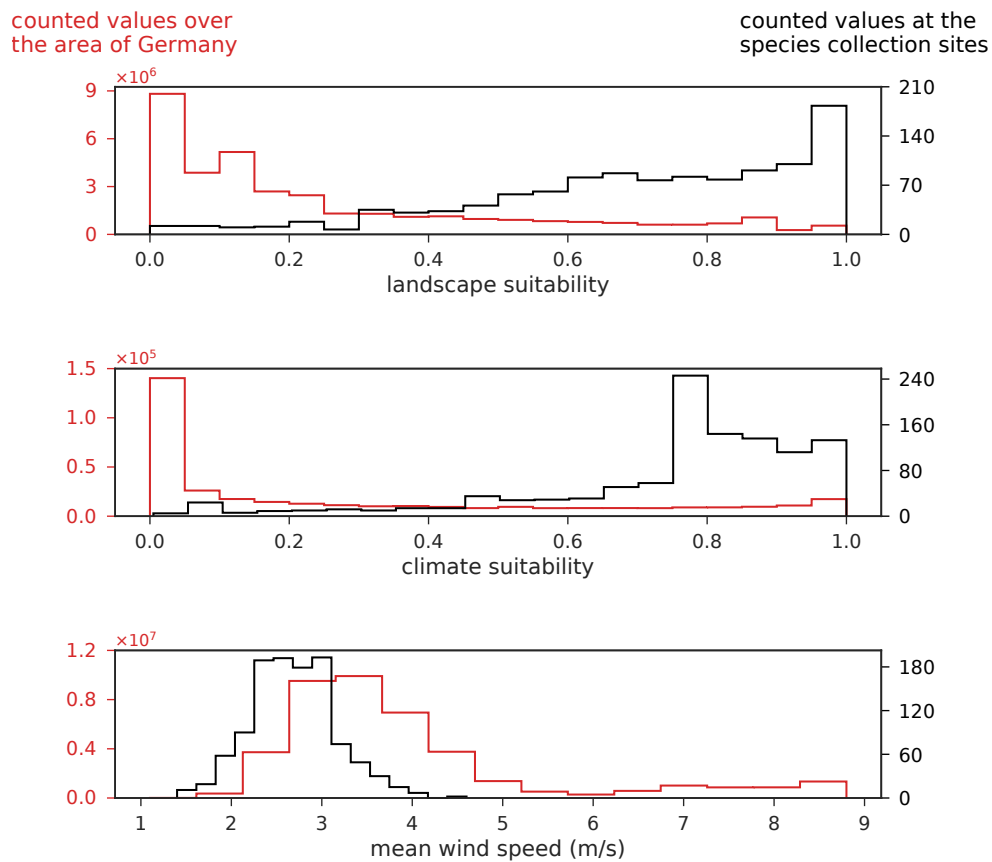
Figure 3.8: Analyses that helped define the fuzzy membership functions. Histograms showing for each input raster array (landscape suitability, climate suitability and mean wind speed) the distribution of values at the field collection sites (black line) compared to the distribution of values of the input raster arrays (red line)

climatic conditions, the largest areas suitable for the mosquito are to be found in central to southwestern Germany. In southeastern Germany, appropriate areas will steadily expand under future climatic conditions. The coastal north, the generally more northern plains and parts of the alpine mountains in the south seem consistently unsuitable for the establishment of the species. Also remarkable is that highly suitable conditions are predicted for regions that are inappropriate according to the climate model input; at the same time, unsuitable conditions are predicted inside climatically suitable regions, e.g. in the southwestern part of the country.

3.4.3 Evaluation of the fuzzy model

In the application of the fuzzy model to the climatically suitable areas of the years 1981–2010, the predictive values, ranging from 0 to 1.0, at the validation points (n = 1110) show a dominance of
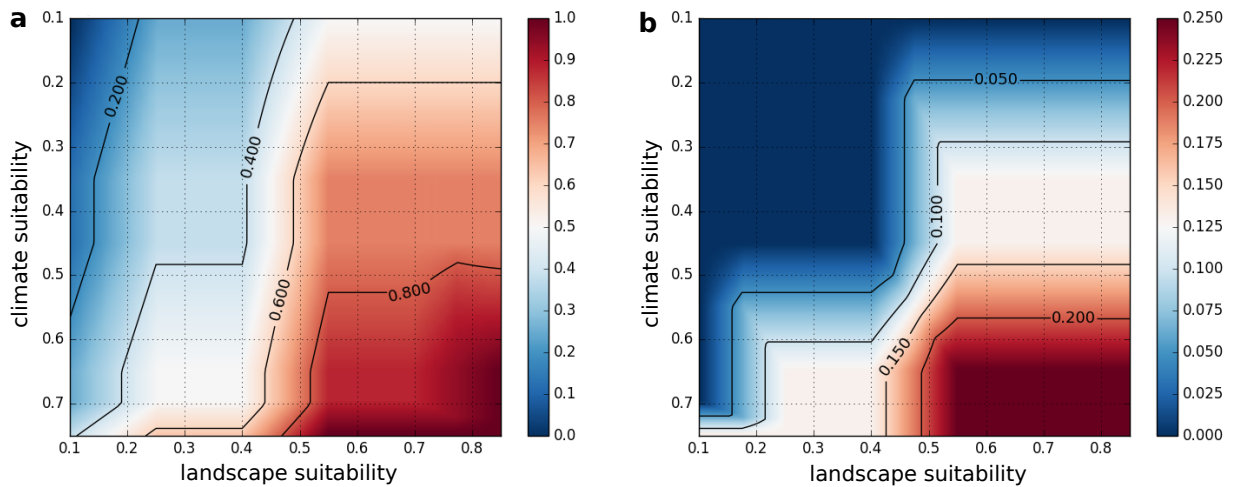
Figure 3.9: Illustration of the influence of wind speed in the model. Outputs of the fuzzy model according to wind speeds of 3 m/s (**a**) and 5 m/s (**b**). The different scales of both graphs illustrate the strong influence of the model input parameter "wind"

1.0 values and a strongly left-skewed distribution (Fig. 3.11). Comparing the fit values of the fuzzy model with those of the input models of landscape suitability and climate suitability (Fig. 3.12), it becomes clear that the fuzzy model explains the occurrence of the species significantly better than the input models. The "exactness" after Früh et al. [17] (average prediction value at all validation points) is 0.86. Small occurrence probabilities with a maximum value of 0.5 still accounted for 10 % of the predictive values at the validation points (Fig. 3.12), yielding a model selectivity [17] of 0.85 ("selectivity" considers the threshold prediction value at 10 % of the lowest predictions at the species collection sites, and reflects the percentage size of the area of Germany that remains unsuitable at this threshold.).
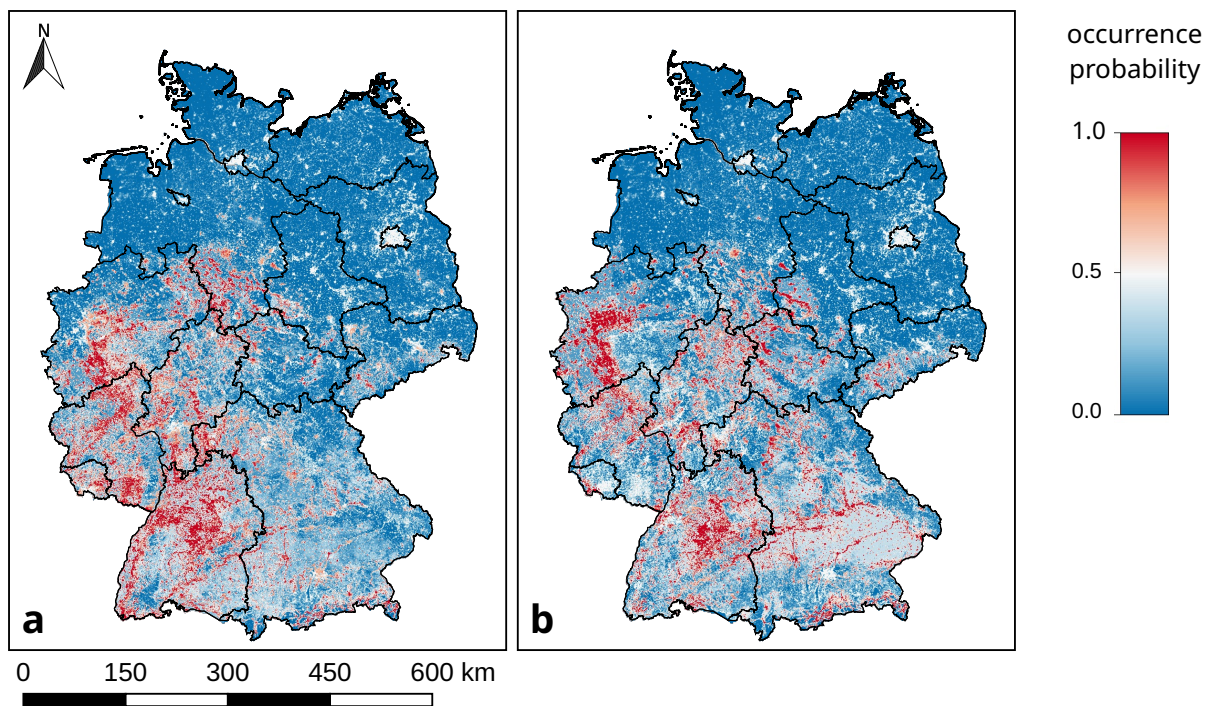
Figure 3.10: Results of model applications for current and future climate conditions. Occurrence probability of *Aedes japonicus japonicus*, depending on our fuzzy model approach applied for climate conditions of the period 1981–2010 (**a**) and the prediction of future climate conditions of the period 2021–2050 (**b**). Projection: Gauss-Krüger zone 3. The geodata of Germany originate from the Bundesamt für Kartografie und Geodäsie [58]
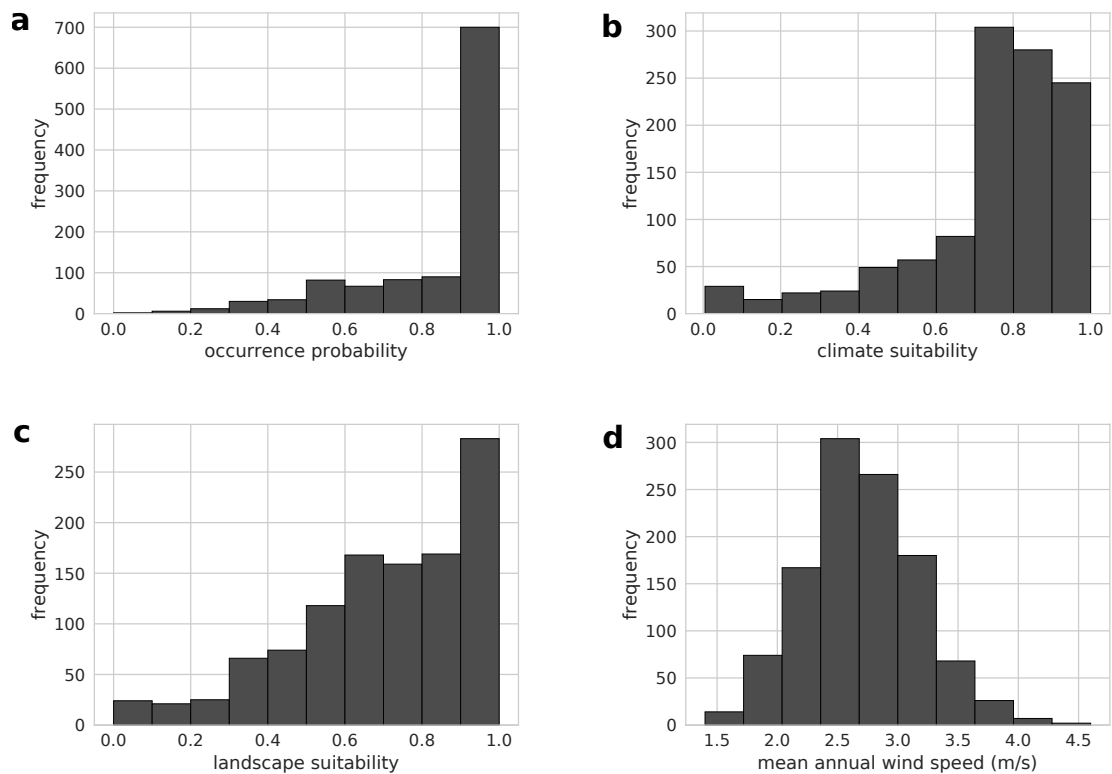
Figure 3.11: Model evaluation. Calculated probability and suitability for the occurrence of *Aedes japonicus japonicus* at the field sampling sites according to the fuzzy model application for 1981–2010 (**a**), the climate model for 1981–2010 (**b**) and the landscape model (**c**). The wind histogram (**d**) demonstrates the mean annual wind speeds for 1981–2010 at the sampling sites. The species sampling data are from the years 2012–2017 (database update 10 April 2018, number of collections = 1110)
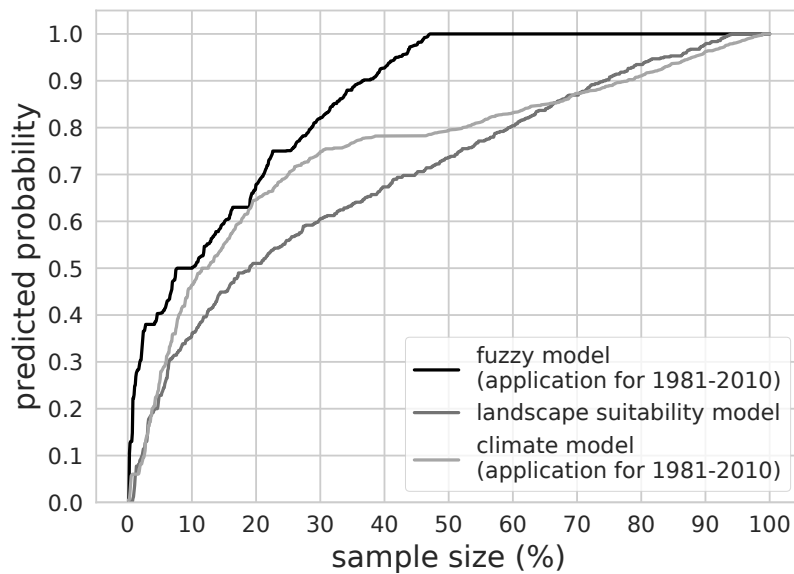
Figure 3.12: Evaluation of the fuzzy model in comparison to the underlying models. Cumulative gain chart showing the calculated occurrence probabilities at *Aedes japonicus japonicus* field sampling sites. The occurrence probabilities are derived from the application of the fuzzy model for the period 1981–2010 (black line) and, for comparison, from the fuzzy model inputs "landscape suitability" (dark grey line) and "climate suitability", depending on the application of the climate model for the same time period (light grey line). The species sampling data are from the years 2012–2017 (database update 10 April 2018, number of collections = 1110)

Table 3.3: Linguistic fuzzy rules

Premises			Conclusion
If the suitability of landscape is...	And if the wind speed is...	And if the suitability of climate is...	Then (linguistic)
low	comfortable	bad	bbbb
low	comfortable	medium bad	bbb
low	comfortable	medium good	bb
low	comfortable	good	b
low	high	bad	bbbb
low	high	medium bad	bbbb
low	high	medium good	bbb
low	high	good	bbb
low	too high	bad	bbbb
low	too high	medium bad	bbbb
low	too high	medium good	bbbb
low	too high	good	bbb
medium low	comfortable	bad	bb
medium low	comfortable	medium bad	b
medium low	comfortable	medium good	m
medium low	comfortable	good	g
medium low	high	bad	bbb
medium low	high	medium bad	bb
medium low	high	medium good	m
medium low	high	good	m
medium low	too high	bad	bbbb
medium low	too high	medium bad	bbbb
medium low	too high	medium good	bbb
medium low	too high	good	bbb
medium high	comfortable	bad	m
medium high	comfortable	medium bad	gg
medium high	comfortable	medium good	ggg
medium high	comfortable	good	gggg
medium high	high	bad	bb
medium high	high	medium bad	m
medium high	high	medium good	g

Table 3.3: Linguistic fuzzy rules

Premises			Conclusion
If the suitability of landscape is...	And if the wind speed is...	And if the suitability of climate is...	Then (linguistic)
medium high	high	good	gg
medium high	too high	bad	bbbb
medium high	too high	medium bad	bbb
medium high	too high	medium good	bb
medium high	too high	good	bb
high	comfortable	bad	m
high	comfortable	medium bad	gg
high	comfortable	medium good	gggg
high	comfortable	good	gggg
high	high	bad	bb
high	high	medium bad	m
high	high	medium good	gg
high	high	good	ggg
high	too high	bad	bbbb
high	too high	medium bad	bbb
high	too high	medium good	bb
high	too high	good	bb

3.5 Discussion

3.5.1 Model validation

The fuzzy model scored very well in the validation regarding the analysis of the species collection sites. Compared to the climate models based on combinations of different machine learning methods [17], the fuzzy model achieved a significantly higher value of exactness (0.86 compared to 0.63–0.65) and selectivity (0.85 compared to 0.63–0.67), while providing a ten times higher model resolution (only the three best results of the mentioned study were used for comparison).

The better performance of our model can be partly explained by the calibration of the climate model, with a larger set of occurrence data being used for training (data for the years 2015–2017 were additionally used, but not those from 2011 which were only few). However, the calibrated climate model scored only slightly better in the validation, the exactness improved by 5–7 % while the data resolution remained unchanged.

We did not calculate the standard model quality parameter of AUC (area under the curve [50]) due to the lack of true absence data in our study. We also decided to not generate any pseudo-absence data for evaluating the model, as such data have a high degree of uncertainty caused by the methodology of collecting the model species [50]. In our case, the collection data are especially unsuitable for identifying unsuitable land use types, as 79 % of the validation data originate from the citizen science project "Mückenatlas", hence the collections are mostly derived from densely populated regions. By contrast, data from the active collections came from deliberately selected sites, in particular cemeteries and private gardens. Forested and agricultural regions are, however, underrepresented in the collection data.

3.5.2   Advantages and disadvantages of the fuzzy model approach

The dependence of the *Ae. j. japonicus* findings on the monitoring method was a strong argument for using a modelling technique that can be directly controlled by the modeller and that is not based on machine learning algorithms. Therefore, we chose the method of fuzzy modelling where biological expert knowledge and field observations could be integrated into the model. The logical approach made the model robust against the discovery of dependencies not existent in reality (e.g. significantly more individuals of the species occur in less densely populated areas than the monitoring data confirm, but this has no effect on the model). The approach, however, also contained sources of error because we were dependent on the availability of biological knowledge about this species which is still incomplete. It is also possible that we misjudged the importance of certain environmental requirements of *Ae. j. japonicus* or, based on the areas investigated, developed a subjective impression of possible landscape suitability, which was incorrect or not applicable throughout Germany (see subsection "Input data" (3.5.3) below).

3.5.3   Input data

The process of creating the dataset of landscape suitability was challenging, mainly because some ecological characteristics of *Ae. j. japonicus* have not yet been determined. This led to uncertainties in the allocation of suitability values for some forms of land use (Tables 3.1, 3.2). Major

uncertainties concerned human settlements and coniferous forests. Regarding human settlements, we have not differentiated between urban and rural settlements. This differs from studies in which significantly more individuals of *Ae. j. japonicus* were found in rural areas compared to urban areas [22, 51]. However, this observation cannot be confirmed for Germany. A large number of collection data linked to the citizen science project "Mückenatlas" was obtained from both rural and urban areas; only centres of larger cities seemed hardly populated. Gardens and discontinuous urban fabrics therefore received a suitability value of 1.0 on a scale between 0 and 1, while a low value of 0.2 was allocated to the category of continuous urban fabric. A residential dataset specifically for rural areas was not integrated into the landscape model.

Also for coniferous forests, little information is available about the habitat suitability for *Ae. j. japonicus*. The biologists among us had different experiences. For example, some observed little to no larvae of the species in coniferous forests within their distribution areas in Germany. If containers (small clay pots) were made available, however, these were colonised. They also reported that larvae usually do not occur in flower vases in populated cemeteries under certain coniferous species, while they can be found under deciduous trees. This is probably because substances in the needles of various species (terpenes and oil) can be detrimental for larvae and pupae of mosquitoes [52, 53]. Therefore, and also because it is unusual that conifers provide cavities that can fill with water compared to deciduous trees [54, 55], we have assigned a low suitability value (0.2) to coniferous forests.

The distance at which land use types influence each other in terms of the potential occurrence of the species has also not yet been widely investigated by biologists. In repeated applications of the sliding window (for the creation of the landscape suitability input map) with different window sizes, the most plausible results were shown for a size of $700\,\text{m} \times 700\,\text{m}$, which corresponds to a radius of around 350 m when looking at the central pixel of the window. Our finding roughly corresponds to the results of flight distance studies of mosquitoes, as summarised by Verdonschot and Besse-Lototskaya [56]. These authors showed that, although the maximum flight distance of an individual of the species can be up to 1600 m, the mean flight distance, measured by mark-recapture experiments, within the genus *Aedes* is only 89 m with a standard deviation of 50 m (to our knowledge, there is no information about the mean flight distance of *Ae. j. japonicus*). Also of interest are calculations of the percentual reduction of the number of mosquitoes with increasing distance when an inhospitable buffer zone is established. *Aedes albopictus*, which is also a container-breeding species in settlements and has similar host preferences, would be reduced by 99 % for a 617 m wide barrier, 90 % for a 347 m wide barrier and 70 % for a 97 m wide barrier [56].

Another reason why the creation of the landscape suitability dataset was challenging was due to difficulties in data acquisition. It is possible that the age structure of deciduous trees could also be taken into account into the landscape model, since young deciduous trees have fewer tree holes than older trees. However, since such a dataset is not available for Germany, we were not able to evaluate this aspect and integrate it into the landscape model. Another problem caused by the availability of geodata was the combination of berry fruit and fruit tree plantations. This category of land use was derived from the CORINE dataset and is problematic, as fruit tree plantations are probably well suited and berry shrub plantations clearly poorly suited habitats for *Ae. j. japonicus*. We have given this category a rather low value of landscape suitability (0.3 within a range of 0 to 1) as there are yet no particular occurrence reports of *Ae. j. japonicus* from within fruit tree plantations (where pesticide application might also have a negative effect on the development of mosquitoes). With an additional effort, the two types of land use could be separated from each other to improve the model, e.g. by satellite image analysis. However, fruit tree and berry plantations cover less than 0.5 % of the total area of Germany, so the unfavourable combination of both forms of land use into one category is not expected to reduce the quality of the model significantly.

Wind as a model input is an interesting novelty compared to previously published climate models for the occurrence of *Ae. j. japonicus* [7, 15, 16, 17]. This factor significantly improves the model. However, it is also a parameter that is dependent on land use. Wind data for Germany are also not exclusively based on measurements but partly on a model that takes into account land use as well as terrain elevation and geographical location. The data relate to 10 m above ground level, but a wind speed map related to a maximum of 5 m above ground would be preferable for our purposes.

The climate model that served as input for this approach shows similar results to other climate models for the occurrence of *Ae. j. japonicus* in Germany under current climatic conditions [15, 16]. Under future conditions, however, the results from [15] differ significantly from ours: a general reduction of suitable areas is predicted, while our forecast indicates a continuing high availability of suitable areas in Germany, only with partially shifted central areas. The difference is probably due to the use of different climate variables and training data of *Ae. j. japonicus* as well as to the application of different modelling approaches. Generally, the estimation of the effects of climate change on the potential distribution of the species is very vague in all approaches, since no regional effects have been considered and the forecasts of precipitation development in Germany vary considerably. Precipitation, however, is of particular importance for container-breeding mosquito species.

Concerning the result of the fuzzy model for the probable future conditions of the years 2021–2050 (Fig. 3.10b) one has to be aware that the aspect of land use change, which in turn might have an influence on wind conditions, is not considered.

3.5.4   Fuzzy rules

The model applications show highly suitable occurrence areas in climatically unsuitable regions, especially in densely populated areas. This is due to the fuzzy rules we have established based on the assumption that an unsuitable climate can partially compensate for a very suitable form of land use. We founded this assumption on the fact that the climate model, which served as input to the fuzzy model, had a high proportion of precipitation variables on all climate parameters, and the climate model classified areas with low precipitation as unsuitable. This is correct for calculating the climatically suitable regions for *Ae. j. japonicus* in Germany, as the species is relatively tolerant to different temperature conditions considering its ecological adaptations to cold regions as well as its occurrence in subtropical to tropical regions (Florida [6], Hawaii [51]) and the Mediterranean region (Spain [10]), in addition to its predominance in cool temperate zones.

However, certain types of land use can compensate for the lack of precipitation, e.g. in residential areas and gardens it can be assumed that people regularly refill flower pots, that rain water barrels and wells are available or that there are irrigation systems. In forested areas, cool air and limited insolation can reduce evaporation, which means that the water in tree cavities probably lasts longer than in other water containers.

Conversely, we assumed that an unsuitable land use type can only marginally be outweighed by a suitable climate in our model, as the absence of certain habitat characteristics make the occurrence of the species considerably more unlikely. For example, there are no breeding and shady resting sites on pastures, which cannot be compensated for by an appropriate climate.

The fuzzy model could be further improved by training procedures as for example applied in Wieland and Mirschel [57]. Another important step would be to build a model that considers the propagation paths and invasion speeds of *Ae. j. japonicus*. Simulation applications are presently being planned for this purpose.

3.6   Conclusions

The paper introduces a nested approach to model the habitat suitability of invasive mosquito species (here *Aedes japonicus japonicus* in Germany). The first step of the approach is to model

the habitat suitability with respect to climate variables using machine learning. The second step is the development of a model that considers regional influences such as land use and the availability of specific landscape elements. For this purpose, the integration of expert knowledge has proven to be useful. In a final step, these models and any further relevant data can be logically combined by means of fuzzy modelling. The nested approach has proven to be very effective in this study. We were able to generate potential distribution maps with a high prediction accuracy and spatial resolution of $100\,\mathrm{m} \times 100\,\mathrm{m}$, which could serve as a basis for the conceptual design of control measures in the event of a disease outbreak mainly caused by the vector activity of *Ae. j. japonicus*. The combination of all parameters could better explain the distribution pattern of the species in Germany than the individual models (climate or landscape only) and data (wind). All model input data, scripts and software are open-source and freely available, so the model can easily be applied to other countries or, more generally, to other species, especially, but not exclusively, within the family of Culicidae.

Authors' contributions   AK and RW were responsible for the conception and design of the model. AK did the literature research, the acquisition, analysis and interpretation of data and drafted the manuscript. MK, DW and HK contributed biological expert knowledge. RW, HK, FH, JMJ and MK critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate   Not applicable.

Consent for publication   Not applicable.

Competing interests   The authors declare that they have no competing interests.

3.7   References

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Chapter 4

West Nile virus model with vector mosquito flight simulator

Linking a compartment model for West Nile virus with a flight simulator for vector mosquitoes

Authors   Antje Kerkow[a,b,c], Ralf Wieland[a], Jörn M. Gethmann[d], Franz Hölker[b,c], Hartmut H. K. Lentz[b,c,d]

a   Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany
b   Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany
c   Freie Universität Berlin, Department of Biology, Chemistry, Pharmacy, Institute of Biology, Königin-Luise-Str. 1-3, 14195 Berlin, Germany
d   Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald - Insel Riems, Germany

4.1 Graphical Abstract

Compartment model   Mosquito flight simulator
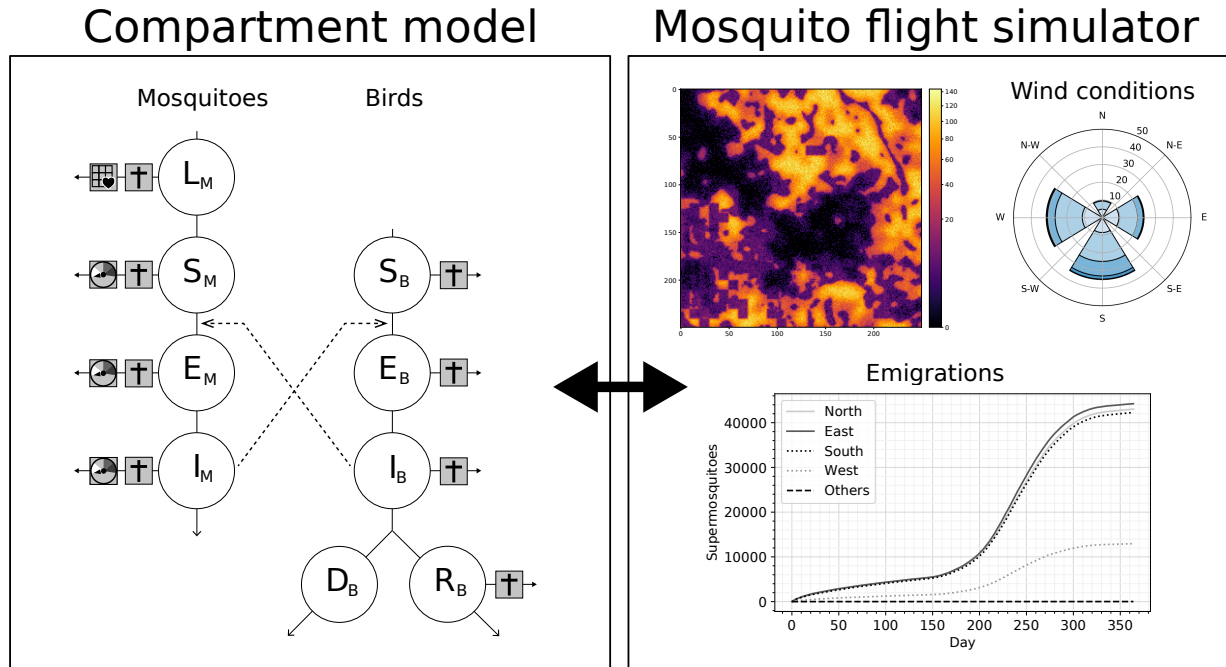
4.2 Abstract

Compartmental SIR and SEIR models have become the state of the art tools to study infection cycles of arthropod-borne viruses such as West Nile virus in specific areas. In 2018, the virus was detected for the first time in Germany, and incidents have been reported in humans, birds, and horses.

The aim of the work presented here was to provide an estimate of the local spread of the virus after its introduction to a new location through the movements of mosquitoes over time and space. For this purpose, we adapted an existing SEIR model for West Nile virus to the conditions in Germany (temperatures, geographical latitude, bird and mosquito species densities) and the characteristic transmission and life trait parameter of a possible host bird and vector mosquito species. We further extended it by a spatial component: an agent-based flight simulator for the mosquitoes. It demonstrates how the female mosquitoes move within the landscape due to habitat structures and wind conditions and how many of them leave the region in the different cardinal directions.

We applied a space-time coupled model to the Eurasian magpie (*Pica pica*) and the Asian bush mosquito (*Aedes japonicus japonicus*), which are both widely distributed in Germany and

discussed as important hosts and vectors, respectively. We also applied the model to three study regions in Germany, each representing slightly different climatic conditions and containing significantly different pattern of suitable habitats for the mosquito species. To answer the question whether the bush mosquito is superior in maintaining the transmission cycle with birds, we compared results from model applications with this species to those of an aggregate of species from the *Culex* genus. The results reveal that, given an equal local abundance, the degree of ornithophilia of the *Culex agg.* must exceed 33 % to compensate for a higher transmission efficiency of *Ae. j. japonicus*.

4.3 Introduction

4.3.1 Background

West Nile virus (WNV), a *Flavivirus* and member of the Japanese encephalitis serocomplex [1], is an arthropod born pathogen maintained in enzootic transmission cycles between birds (amplifying hosts) and ornithophilic mosquitoes (vectors) [1, 2]. Humans, horses and other mammals are dead-end hosts that may be infected but do not develop viraemiae high enough to contribute in maintaining the transmission cycle. While most human infections are asymptomatic, about 25 % of those affected develop symptoms such as fever and headache. Less than 1 % develop severe neurological disease comprising meningitis, encephalitis and paralyses or succumb to the disease [2–5].

The virus is native to Uganda, from where it has spread to Eurasia, Australia and the Americas [1, 3, 6]. At least 8 evolutionary lineages have developed, of which the lineages 1 and 2 are most relevant for humans [1, 7]. In the Americas, so far only lineage 1 has occurred and was responsible for particularly intense outbreaks in 2002 and 2003 in the US with together more than 14,000 reported human cases, of which nearly 6000 were neuroinvasive and 998 fatal [8, 9]. The epizootics in the US caused high death rates among birds, especially American crows. Its population sizes were reduced by up to 90 % in some areas [9].

In Europe, since the introduction of WNV-Lineage 2 by migratory birds from the sub-Saharan region in 2004, both variants of the virus are circulating. In contrast to the situation in the US, the formerly sole substrain of lineage 1, which had been distributed in the Mediterranean region, was characterised by very low pathogenicity for birds and moderate pathogenicity for humans and horses [10, 11]. Lineage 2 has spread further through the intercontinental movements of birds [10, 12]. It caused outbreaks in Hungary, Austria, Greece, Serbia and Italy with numerous

neuroinvasive cases in humans and horses. Various bird species were found with antibodies or succumbed due to the disease [4, 7, 10, 13, 14].

Warm temperatures are a requirement for WNV outbreaks because the virus replication time in the mosquito is shortened as the temperature rises, thus increasing the probability of transmission with the next blood meal [15]. This is why in temperate and Mediterranean regions epizootics in bird populations only occurred during the summer months [16]. In 2018, Europe experienced an intense and long heat wave, bringing the continent its worst epizootics and epidemics to date. Many countries were affected for the first time, including eastern Germany with 12 detected infections in birds and 2 in horses [12, 17, 18]. In 2019 and 2020, there were repeated cases in the affected regions in Germany, which leads to the assumption that the virus hibernated in local mosquito populations [17–20].

Host birds

Many bird species seem susceptible to the virus as antibody tests and RNA-extractions show [21], but only some of them develop viraemiae and death rates high enough to make them an important host and sentinel species [22, 23]. In North America, especially American crows (*Corvus brachyrhynchos*), American black- and yellow-billed magpie (*Pica hudsonia, P. nutalli*) and other corvids were excessively found dead and with high viraemiae [24, 25].

In Germany, the number of proved cases in birds is still low. Infections have so far been detected e.g. in the wild species Northern goshawk (*Accipiter gentilis*), common blackbird (*Turdus merula*), Eurasian blue tit (*Cyanistes caeruleus*), Alpine chough (*Pyrrhocorax graculus*) and in caged owls (*Strix nebulosa*, *Bubo bubo*) [12, 19, 26].

According to reports from European countries, birds of prey and corvids were observed to have high mortality from WNV-2. The most affected species seem to be Northern goshawk (*Accipiter gentilis*), house sparrow (*Passer domesticus*), Eurasian magpie (*Pica pica*) and hooded crow (*Corvus cornix*) [7, 11, 27–31].

Based on field studies in Spain, Napp et al. (2019) [31] propose the magpie as a sentinel species. The magpie is one of the most common corvid species in Europe, sedentary and showed high virus susceptibility, virus titres and mortality in experimental studies [25].

Vector mosquitoes

WNV has been detected in numerous mosquito species in nature worldwide, especially in species of the genera *Culex* and *Aedes* [32, 33]. In Europe, the main vectors are supposed to be mosquitoes of the widespread *Cx. pipiens* complex including the two biotypes *Cx. p. pipiens* and

Cx. p. molestus, as well as *Cx. torrentium* and *Cx. modestus* [34–37]. Besides those, *Ae. vexans* and *Ae. japonicus japonicus* are also considered species [17].

Especially the potential future role of the Asian bush mosquito *Ae. j. japonicus* in WNV transmission is currently hotly debated [17, 38]. Laboratory experiments with specimens collected in Switzerland and the US showed that it may have a significantly shorter virus incubation period [39] and significantly higher dissemination and transmission rate than *Cx. pipiens sl.* [39–42]. But there is also a laboratory study with individuals from Germany that showed a resistance to infections with West Nile virus [43].

Aedes j. japonicus is an invasive species in Europe and detected in Germany since 2008 [44]. To date, it is mainly established in the western part of the country where, to our knowledge, WNV has not yet appeared [17]. Its imagoes are typically longer active in the year than those of other mosquito species [45–48], and the larvae are often found in extremely high densities. In some areas, *Ae. j. japonicus* is even the most common mosquito species according to larval collections [38, 47].

4.3.2 Previous models and motivations

Because of its medical relevance for people for whom vaccines are not yet available as well as for the poultry industry and the protection of wild bird populations, it is highly important and indeed urgent to take precautionary measures in the potential WNV risk areas. Models shall help to understand the complex interrelationships that contribute to the establishment of an epizootic, identify high-risk areas, and assess the effect of precautionary measures.

So far, WNV models focused either on the spatial or the systemic component. The spatial models identify potential risk areas by modelling the habitats of vector mosquitoes [49–51], host birds [52] or an overlap of both [35]. Other spatial approaches aim to identify WNV risk areas by analysing the climate and other spatial features of areas where epizootics already occurred and apply machine learning methods such as supervised learning [53–58].

Compartment models based on differential equations, on the other hand, are much more complex and focus on the systemic component. They consider both seasonal and temperature-dependent population densities of host birds and vector mosquitoes and simulate the development of the infection processes after virus introduction in a given region. Since about 20 years these models have been successfully used to study arbovirus infection cycles like that of WNV [59–62]. The models are called SIR or SEIR models, which refers to the infection stages in which the model organisms can be found: "Susceptible", ("Exposed"), "Infected-infectious" and, only valid for the vertebrate hosts, "Recovered and Immune". They assume a homogeneous distribution of

host birds and vector mosquitoes in a given outbreak region and are usually calibrated by means of the number of encountered dead birds [61, 63].

4.3.3   Knowledge-gaps

Due to the assumption of homogeneity in the previous compartmental WNV models, it was on the one hand not possible to locate the risk areas more precisely and on the other hand to estimate the spread of the virus within the region and beyond. To achieve this goal, one needs a spatial component that considers the locations and movements of the animals. In the work presented here, we will extend a compartment model for WNV by a spatial component.

We further discovered that in existing compartmental WNV models [60, 61] the calculation of an unknown parameter in the infection equations, the vector population size in the epidemic area, is problematic. This is because the contact rate of vector mosquitoes with host birds has a huge impact on the equations, but is solely derived from observations of temperature-dependent blood digestion. The relation to the blood diestion is plausible. However, it was not considered that the vector mosquitoes integrated in the model (an aggregation of *Culex pipiens sl.* and other *Culex* species, especially *Cx. tarsalis*) do not feed exclusively on the host birds with every required blood meal. Other birds as well as mammals, amphibians and reptilians are, for example, also used by both biotypes of *Cx. pipiens* [64]. As a result of this set-up, the population density of mosquitoes is calculated to be significantly too low, in our opinion. Conversely, if the model is applied to new epidemic areas with only a few case numbers and if mosquito densities confirmed by monitoring campaigns are integrated, the model will considerably overestimate the course of infection due to the high contact numbers. The problem becomes even more apparent when the model is applied to the mosquito species *Ae. j. japonicus*, which is mammophilic and therefore seeks out humans and mammals more frequently than birds for its blood meal. The model thus needs a factor for the proportion of mosquito blood meals taken on the host birds.

4.3.4   Objectives

We aimed to provide a model that, after introduction of the virus into a new area, can (i) predict the potential regional extent of an epizootic based on realistic density data of host birds and amplifying vector mosquitoes, (ii) identify high-risk transmission hotspots within the region, and (iii) estimate the local spread of the virus into surrounding areas by mosquito emigration. The latter two model features are intended to support targeted mosquito control measures to contain the virus.

We focus on the local occurrence and movements of vector mosquitoes to estimate the virus spread because it can be effectively controlled by decimating mosquito populations. On the wild bird side, no vaccination exists so far and population control would be difficult to establish and not be a socially accepted measure. Furthermore, the active (autonomous) movements of mosquitoes are usually of smaller distance and more predictable than those of the vector birds. We also do not consider virus cross-overs to humans and horses, only the trans-sylvatic transmission cycle is studied.

Additionally, we had the objective to answer the questions whether the vectorial capacity of *Ae. j. japonicus* is comparable to that of the *Culex agg.* (species aggregation as used in the models of [61] and [63]) and whether *Ae. j. japonicus* is able to maintain the trans-sylvatic transmission cycle without the presence of other host species, provided the results from the laboratory studies in Switzerland mentioned above are representative.

4.3.5   Model implementation and applications

For implementation, we numerically adapted an existing model for WNV that was originally implemented for Montreal in Canada [61] and itself is based on a USUTU model for a region in Austria [63]. We then extended it by a spatial component, a flight simulator for vector mosquitoes.

The flight simulator is based on the agent-based simulation technique. Agent-based models (ABMs) have successfully been used for the dispersion modelling of insects and other mosquitoes [65–67]. The advantage of this type of model is that it can represent environmentally induced behaviour changes, random decisions, interactions between individuals (the agents) and agent heterogeneity [68].

The space-time coupled model is applied to 3 study regions in Germany with a size of $625\,\mathrm{km}^2$ each, but a considerably different number and distribution of vector mosquito habitats and slightly different climatic conditions. The host birds in the model are represented by the Eurasian magpie (*P. pica*), and the vector mosquitoes by the Asian bush mosquito (*Ae. j. japonicus*). The latter was used for the development of the spatial component because a well validated, model-based habitat map for the species in Germany already exists. Due to its high resolution, it is a suitable basis for flight simulations and local density estimates. The magpie, on the other hand, was integrated into the model not only because of its host characteristics described in Chapter 4.3.1, but also because the species occurs in urban habitats, especially in parks, cemeteries, allotment gardens, as well as in semi-open and open landscapes [69]. Therefore, its habitat may overlap with that of *Ae. j. japonicus* to a large extent [70], principally allowing for frequent contacts with the mosquito species.

To answer the question of which mosquito species has a higher vectorial capacity and therefore contributes the most to the development of an epizootic, we applied the model without spatial coupling separately for *Ae. j. japonicus* and the *Culex agg.* and compared the results.

4.3.6   Summary of model innovations

Our work presents the following innovations to existing WNV models:

 (i) a numerical adaptation that considers mosquito blood meals on organisms other than host birds included in the model. This is useful for the integration of real density data of vector mosquitoes and thus enables the model to be applied to regions where the virus has not yet circulated for a long time and where case numbers are still low.

(ii) the extension by a spatial component, an agent-based flight simulator for vector mosquitoes which illustrates the possible regional distribution and, taking into account the habitat structure, simulates the self-motion of the mosquitoes and estimates how many of them would probably leave the region towards which cardinal points.

4.4   Materials and Methods

Our model is freely available under the Creative Commons Attribution - Non Commercial 4.0 (https://github.com/akerkow/bush-mosquito-flight-simulator) and implemented in Python 3.7. It has two components that interact with each other. These are, on the one hand, a spatial component that describes the occurrence and movements of female mosquito imagoes in the study regions. On the other hand, there is a temporal component, an adaptation of the SEIR model of Laperriere et al. (2011) [61], that describes the population dynamics of host birds and vector mosquitoes by means of temperature- and seasonal-dependent ordinary differential equations and calculates the proportions of the mosquito and bird specimens in their respective infection stages. The temporal resolution of the model is one day.

The spatial component is based on a two-dimensional habitat suitability map for the potential vector mosquito *Ae. j. japonicus*. The map has been developed using a nested modelling approach consisting of (i) a part that models the global, climatic requirements [71–73] and (ii) a separate part that integrates local land use requirements and the influence of wind strength [70]. It covers the area of Germany, has a resolution of $100\,\text{m} \times 100\,\text{m}$ and shows the habitat suitability as values between 0 and 1. The grid map can be freely downloaded under the link "https://doi.org/10.4228/zalf.dk.90". Due to its high score in evaluation as measured by the hit rate of data that were not

included in a model training, we assume that it correlates with the larval density in areas where the invasive species is already established and widely distributed since several years.

4.4.1 Study regions

We selected three subregions of the grid based habitat suitability map with sizes of $625\,\text{km}^2$ ($25\,\text{km} \times 25\,\text{km}$) for model applications (Fig. 4.1, 4.2, 4.3). In each of them, the occurrence of *Ae. j. japonicus* has been documented for several years [44, 74–77]. In areas that include our study regions 2 and 3, the species has spread rapidly and the larval numbers on the sampling sites have increased dramatically since the first evidence. Region 1 appeared to have a lower spreading potential and larval numbers at the collection sites were partially declining.

a) Region 1   b) Region 2   c) Region 3

Sentinel-2 cloudless layer for 2018 provided by the European Commission, Rendering ©EOX
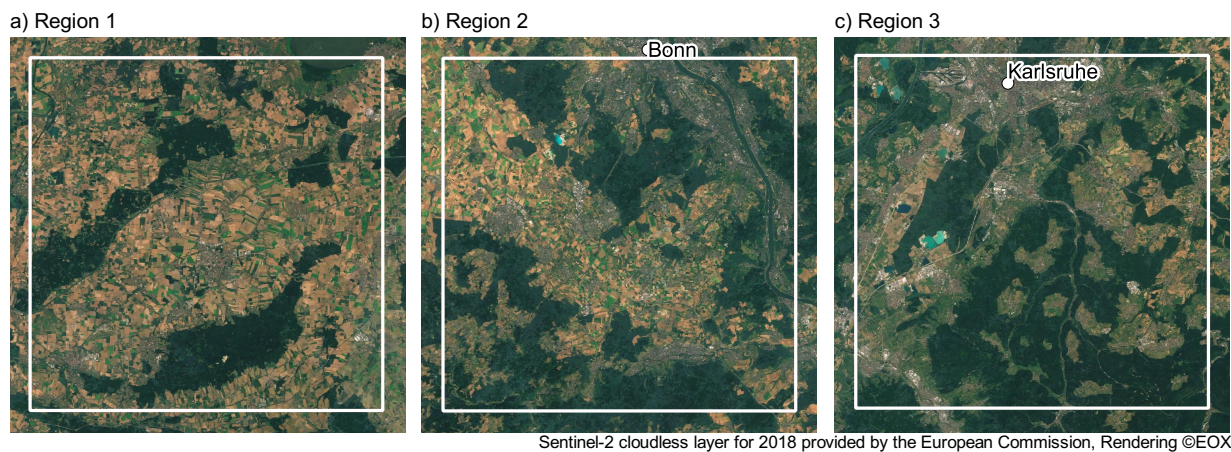
Figure 4.1: Landscape of the study regions with sizes of $25\,\text{km} \times 25\,\text{km}$.

The average habitat suitability of the selected regions for *Ae. j. japonicus*, calculated by Kerkow et al. (2019) [70] are 0.35, 0.66, and 0.79 for Region 1, 2, and 3, respectively (Fig. 4.2). The regions differ not only in their habitat suitability, but also in their climate conditions. The southernmost region lies in the Upper Rhine Valley and is, due to its geographical location, a particularly warm region of Germany (Fig. 4.3).

In the following, the model will be described using the ODD (Overview, Design concepts and Details) protocol [78]) for agent-based models.

4.4.2 Purpose

The model simulates the population dynamics for a WNV vector mosquito and host bird species for several study regions in Germany and a given time period, considering the vector density, local
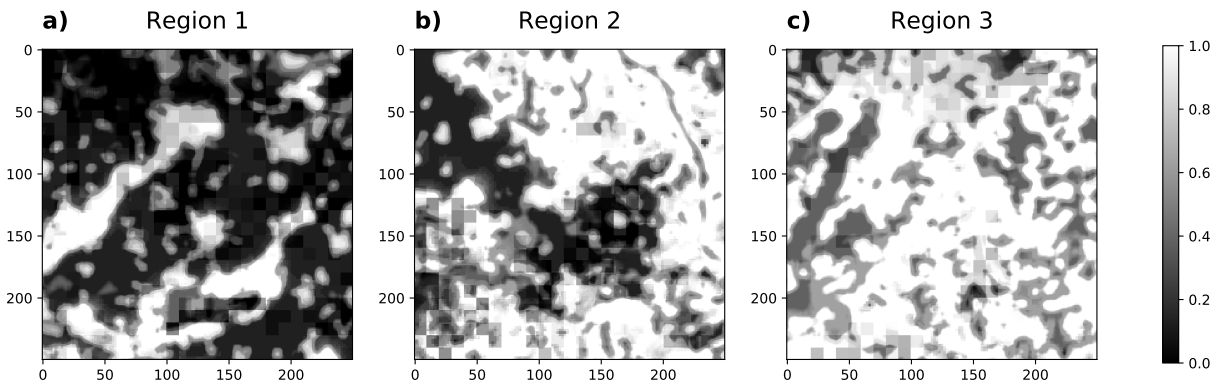
Figure 4.2: Study regions and their habitat qualities for *Aedes japonicus japonicus* [70]) on a scale from 0 (poor quality) to 1 (high quality).

daily mean temperature and time of the year. By supplying small numbers of infected birds, the outbreak of an epizootic is triggered and its possible extent calculated. By means of the spatial simulation, we investigate the impact of different landscape structures on the mosquito population and estimate the daily emigration towards the different cardinal points.

4.4.3 Entities, state variables, and scales

Since only female mosquitoes ingest blood meals and maintain a trans-sylvatic transmission cycle between mosquitoes and birds, only these are integrated both numerically and spatially in the model. Vertical virus transmission from mosquito females to offspring as well as horizontal transmission between birds is neglected in the model as implemented in Laperriere et al. (2011) [61].

Entities in the temporal component

In the temporal component, entities are Eurasian magpies, female mosquito imagoes and female mosquitoes in pre-imaginal, aquatic stages. The mosquitoes belong to *Ae. j. japonicus* or species of the *Culex aggregate*. For the sake of simplicity, we call the imagoes "mosquitoes" and the mosquitoes in the pre-imaginal stages "larvae" as it is done in Laperriere et al. (2011) [61]. The mosquitoes develop from the larvae, whose number is initially determined by the average estimated habitat suitability of the region. They can be in the infection stages "virus susceptible", "exposed" (after contact with infected bird) or "infected and infectious" (Fig. 4.4). Both, larvae and mosquitoes have general temperature-dependent mortality rates but cannot succumb to infection. The model neglects the possibility of vertical transmission. Hence, the larvae have no infection
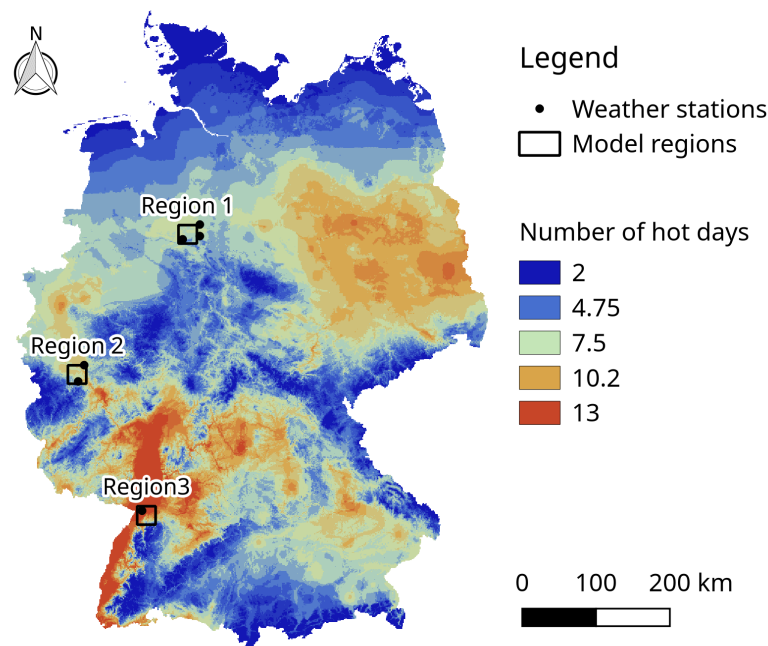
Figure 4.3: Location of the study regions, their nearest weather stations, and illustration of the number of local hot days defined by a maximum air temperature higher than 30°C (annual mean of the long-term period 1981-2010). Geodata source: German Weather Service.

status and always evolve into susceptible mosquitoes. The birds may be in the infection stages "susceptible", "exposed" (after contact with an infected mosquito), "infected and infectious", "dead" (succumbed to infection) or "recovered and immune" (Fig. 4.4). New-born chicks are always virus susceptible.

In addition to temperature-dependent birth and mortality rates, the mosquitoes in the temporal component are characterised by a transmission probability after the ingestion of infected blood, a biting rate, the proportion of blood meals taken from a bird, and their active time during the year. The host birds in the temporal component are also characterised by a daily mortality and birth rate. The mortality rate is solely derived from the average life expectancy of Eurasian magpies and the birth rate on the season, which means the day of the year. Furthermore, the birds have a virus-induced death rate, an incubation rate and a probability of becoming infectious after the bite of an infectious mosquito.

Entities in the spatial component

In the spatial component, the female mosquitoes are displayed individually, but bundled to super-mosquitoes. For every model application the number of mosquitoes forming one super-mosquito
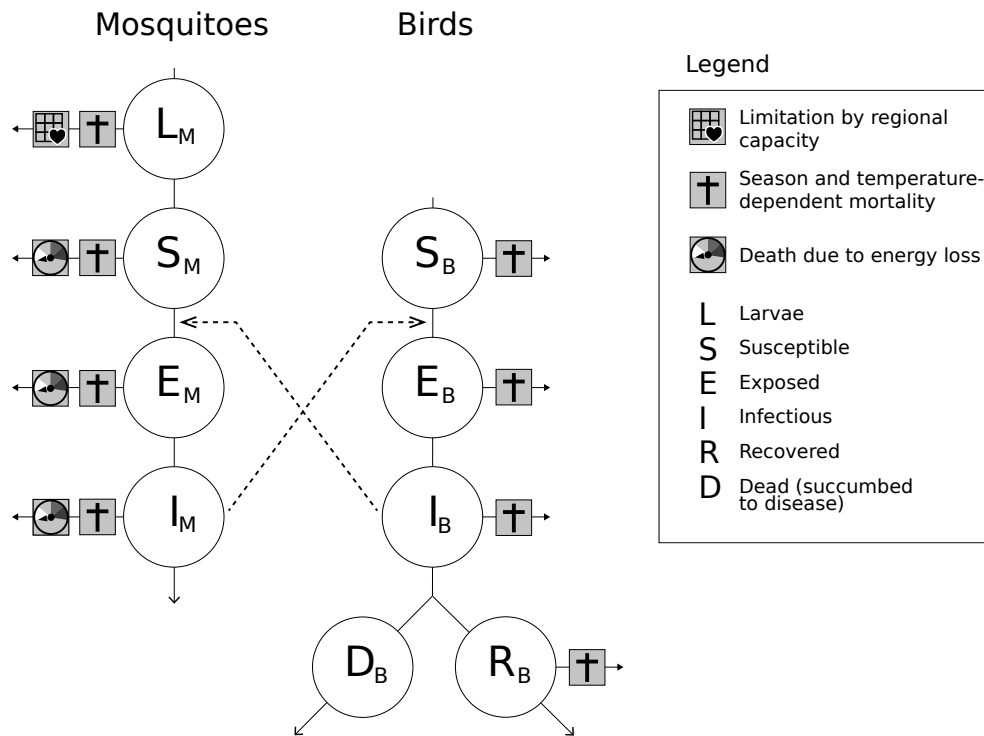
Figure 4.4: Possible chain of infection stages that vector mosquitoes and host birds in the model may undergo; The crosses symbolise the mortalities to which the animals are subject at each stage of infection depending on season and temperature. The dashed arrows symbolise the virus exposure of mosquitoes and birds after contact with an infectious animal of the other species. On the mosquito side, the influence of the spatial model extension (the flight simulator) becomes visible by the determination of the number of larvae and the energy-related mosquito mortality.

can be freely chosen. For the visualisation of infectious mosquitoes, the factor should not exceed 100, for the visualisation of all mosquitoes, however, a factor of 1,000 is advisable to keep the computing time at an acceptable level. The birds are not implemented on an individual level in the model and not exactly located. We assume the vector birds to move widely in comparison to the mosquitoes and being equally present throughout.

The super-mosquitoes are characterised by their position in space (row and column of the grid), an energy level ranging from 1 to 100, and the stage of infection. They are either infectious (I_M) or not infectious (S_M or E_M). In addition, it is recorded whether a mosquito tried to leave the region during a model run and if so, in which cardinal direction. The mosquitoes have a certain motivation to move based on the local habitat characteristics (for every $100\,\text{m} \times 100\,\text{m}$ cell) within the study regions. Moreover, regional prevailing wind directions slightly influence their movements. The super-mosquitoes constantly lose energy every day to a small extent, and a

potentially large amount due to long-distance flights. The energy can be recharged during stays in good habitats. If a certain threshold value is exceeded, they die.

4.4.4 Process overview and scheduling

A main simulation calls both, the temporal component (SEIR model) and the spatial component (flight simulator), and synchronises the mosquito population size and the number of infectious mosquitoes (Figure 4.5): At each time step, first the number of mosquitoes and birds in their respective infection stage is changed by the daily birth and death rates implemented in the time component. The time component uses the information stored by the main model for the previous day for the calculations. The number of mosquitoes is then communicated to the spatial component, which may reduce it due to deaths caused by low energy levels after the spatial movements and in turn communicate to the main component.

At the beginning of the simulation, the temporal component uses initialisation parameters. At this point, the spatial component already plays a role as the carrying capacity of the larvae is derived from the mean habitat suitability of the study region.
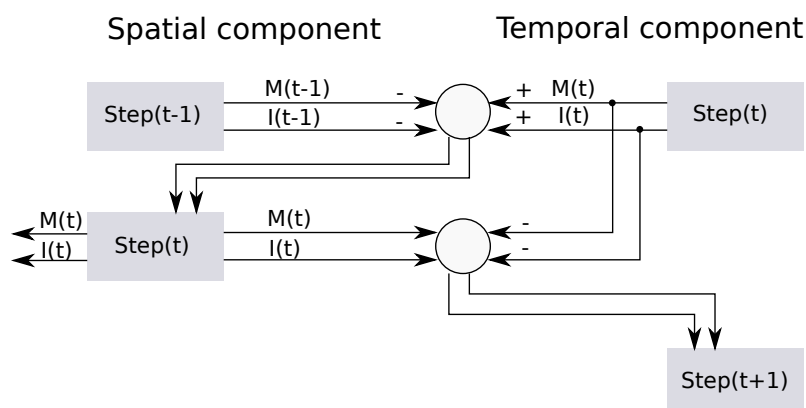
Figure 4.5: Stabilisation of the mosquito simulation by communication between the model components (M = all mosquitoes, I = infectious mosquitoes, t = day of the simulation).

4.4.5 Design concepts of the spatial component

Basic principles

On the one hand, the flight simulator is intended to provide a realistic assessment of the spatial distribution of mosquitoes in a region. On the other hand, the flight distances and routes, which are influenced by habitat structures (ecological corridors, stepping stones and barriers) and partly

by wind conditions, are analysed. The final goal is to determine the possible distribution and spread of the WNV. The flight simulator is a very simple ABM, which has been implemented in such a way that it has an acceptable runtime despite a large number of mosquito agents.

Emergence

There is no emergence in the sense that the mosquitoes influence each other in their movements. The mosquitoes move randomly with the motivation and distance being influenced by the habitat quality of the starting point. The presence of other mosquitoes and the conditions at the possible destinations are not considered.

Adaptation and objectives

The behaviour of the single mosquitoes (which can be bundled to super-mosquitoes) has some stochasticity and depends on the habitat characteristics of their location. If the habitat is well suited, the mosquito will most likely remain there or not fly far. In cells with medium habitat quality, it mostly stays there, but sometimes flies further to meet its needs. If the mosquito is in an unsuitable habitat, it is more likely to fly long distances than in habitats with medium suitability. This enables the mosquito to leave this location more quickly.

An adjustment of the flight behaviour is only provided in closed model mode, where an initiated flight that would end outside the model region does not get executed. A behavioural adaptation of the mosquitoes to explicitly reach a certain objective is not included in the model.

Learning, prediction and sensing

Mosquitoes in the model are not able to learn and cannot predict any future consequences of movements or environmental conditions. They also have no awareness of their condition or that of other agents.

Interaction

Interaction only exists in so far as the mosquitoes visit birds for their blood meals. However, since we assume an equal spatial distribution of the birds, the proportion of infected mosquitoes and birds caused by the infection forces is only numerically calculated and then the infectious mosquitoes are added to or removed from the spatial flight simulation.

Stochasticity

The movement distances of the mosquitoes are random, following Gaussian normal distributions for mosquitoes in cells with medium and high habitat suitability. This means that the mosquitoes stay in a cell relatively often and with decreasing probability longer distances are covered. Also, the direction of every movement is randomised.

If the time component forces a reduction in the number of mosquitoes, they will be removed from the spatial component at random locations as well. This situation happens after the mosquito population reached its maximum size in summer. Finally, the initial spatial distribution of mosquitoes and the location of newly hatched mosquitoes during the simulation are also random, but based on the habitat suitability map.

Collectives

For runtime reasons, the mosquitoes are bundled together to form super-mosquitoes which only unite individuals with the same infection status. Note that the flight simulator only distinguishes between infected and non-infected mosquitoes. Targeted swarming, for example for mating, is not implemented in the model.

Observation

For each time step, the number of larvae as well as the number of mosquitoes and birds in their respective infection states and the number of mosquitoes that have emigrated (open mode) or tried to emigrate at least once (closed mode) in the respective directions can be displayed (Chap. 4.4.8). For this purpose, the data are stored in a csv-file during the simulation. Furthermore, the spatial distribution of all mosquitoes and the infected mosquitoes is stored in NumPy arrays. Using these, we display (i) the movements of the mosquitoes during the entire simulation period in a video and (ii) the distribution of the mosquitoes on the day with the highest infections in a single map. The video was created by means of the Matplotlib animation module.

4.4.6   Initialisation

Mosquitoes and birds in the temporal component

The initial conditions and initialisation parameters of the temporal component are listed in Table 4.1. Our model applications start on January 1st and we assume that no infection has yet been introduced into the region, so there are no virus-exposed and infected birds or mosquitoes and

no birds that are immune. As in the model applications of Laperriere et al. (2011) [61], the bird population is set to the maximum, and the minimum number of mosquitoes required to prevent extinction is 15 % of the carrying capacity of the larvae. Since it is winter, all mosquitoes are assigned to the status susceptible. The carrying capacities for the birds and larvae were determined based on the following considerations:

Table 4.1: Initial conditions and initialisation parameters

Term	Value	Description
K_B	8,226	Carrying capacity of host birds (magpies)
$K_{M_{max}}$	19,900,000	Carrying capacity of mosquito larvae in a perfect habitat
$K_{M_{R1}}$	6,965,000	Carrying capacity of mosquito larvae in Region 1
$K_{M_{R2}}$	13,134,000	Carrying capacity of mosquito larvae in Region 2
$K_{M_{R3}}$	15,721,000	Carrying capacity of mosquito larvae in a Region 3
$N_{M_{min}}$	$K_M \times 0.15$	Minimum number of mosquitoes
S_B	K_B	Amount of susceptible birds
E_B	0	Amount of birds exposed to WNV
I_B	0	Amount of infectious birds
R_B	0	Amount of recovered (immune) birds
L_M	0	Amount of mosquito larvae
S_M	$N_{M_{min}}$	Amount of susceptible mosquitoes
E_M	0	Amount of mosquitoes exposed to WNV
I_M	0	Amount of infectious mosquitoes

Carrying capacity of host birds   The Eurasian magpie is a bird species that is widespread in Europe and very abundant in Germany. With the evaluation of the last monitoring of breeding birds in Germany [69], it was estimated to have a Germany-wide population of 370,000-550,000 territories. A territory contains one breeding pair each. However, a certain percentage of magpies do not breed. The percentage is fluctuating and corresponds to about 14 % per territory [79], which has to be added to calculate the total stock. In order to estimate the bird density in our 625 km^2 large study regions, it must be noted that the relationship between the size of the mapping area and the bird density is not linear because they are provided in categorial classes. For the area of 35,751 km^2 in Baden-Württemberg, for example, the German atlas of breeding birds [69] gives a density of 55,000–70,000 territories. This corresponds to an average of 62,500 breeding pairs, which is 1.7/km^2. The resolution of the distribution map in the breeding bird atlas is about 31 km^2. On this scale, the average density of territories is 8.3/km^2. For the 625 km^2 large study

regions we have assumed, based on the given data, a density of 6.15 territories/ km², which makes approximately 8,226 magpies / 625 km².

Carrying capacity of mosquito larvae To estimate the carrying capacity of the mosquito larvae from *Ae. j. japonicus* for any model region in Germany, we first studied the literature to find the maximum density in a perfect habitat in Central Europe. We then adjusted this value to the size of the model region and multiplied it by the mean percentage habitat suitability according to Kerkow et al. (2019) [70].

To determine the highest possible population density, we considered a study in the Netherlands [80], in which several sample plots were examined in large allotment garden complexes where *Ae. j. japonicus* has been established for several years. Allotment gardens are ideal habitats for *Ae. j. japonicus* due to their rich vegetation structure, irrigation systems and the presence of numerous suitable breeding habitats (rain barrels, flower pots etc.). All possible breeding habitats were counted in the study and the larvae identified. At the time of the highest larval density (September 2015), 242 containers on an area of 6.8 ha contained 4,335 larvae of *Ae. j. japonicus*. This results in 63,750 larvae/ km², of which an estimated 31,875 are female, assuming an equal distribution of the sexes. Based on these observations, we have set the maximum carrying capacity $K_{M_{max}}$ to 19,900,000 for our study regions, which cover 625 km².

We do not know the suitability of the regions for the relevant species of the *Culex aggregate*. For this reason, we concentrate on the transmission characteristics, the occurrence in the year, and the degree of ornithophilia. Hence, we set the carrying capacity for the larvae of the *Culex agg.* to the same number as for *Ae. j. japonicus*.

Mosquitoes in the spatial component

The grid-map in the spatial component is filled up with the number of mosquitoes transferred from the temporal component. The mosquitoes hatch at random locations within the region, but with a higher probability in areas with comparatively high habitat suitability (the larvae are not spatially located).

To achieve this, we have created copies from the habitat maps and reduced its spatial resolution to 500 m × 500 m (in the following, we refer to 100 m × 100 m units on the map as *cells* and larger areas measuring 500 m × 500 m as *patches*). The values of all individual habitat qualities within each 25 ha patch were summed up and again scaled to values between 0 and 1 for each study region. The mosquitoes first hatch on the low-resolution map. A weighted random generator ensures higher hatching rates with increasingly better habitat suitability. The mosquitoes are then

transferred back to the original map with $100\,\text{m} \times 100\,\text{m}$ resolution. The allocation within each $25\,\text{ha}$ patch is random. All newly born mosquitoes start with the maximum energy level of 100.

4.4.7   Input data

The spatial component is driven by means of daily average temperature data and the day length. Temperature data was obtained from the German Weather Service. We chose one to three weather stations close to every study region (Fig. 4.3) and, if they were several, calculated the average temperature. Fig. 4.6 shows the temperature curves for the period from 2018 to 2020, which we used for our long-term model applications. Since the model was applied in 2020, the weather data was only available and integrated until November 7, 2020. The temperature values are smoothed with a weighted average filter and a window size of 2.
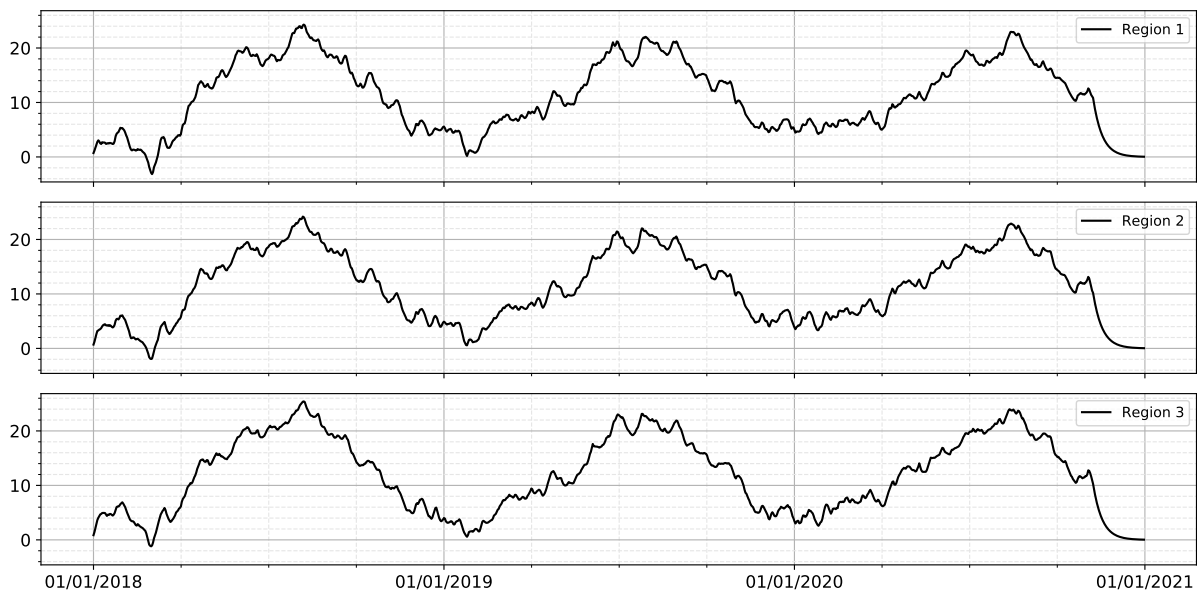
Figure 4.6: Daily mean temperature in the study regions.

For the calculation of the day lengths, we apply the Brock model [81, 82] which maps the latitude (l) of the location and the day (d) of the year on the day length as follows:

$$D(l,d) = 2\frac{cos^{-1}\left(-tan(l)tan\left(23.45 sin\left(360\frac{283+d}{365}\right)\right)\right)}{15} \tag{4.1}$$

Our study regions are located at latitudes 48.9, 50.6 and 52.4. We run all model applications by the average day length of the regions of 50.6. Tests showed that the slightly different latitudes

of the regions do not lead to any difference in the model results regarding the number of infected birds and mosquitoes. Fig. 4.7 shows the calculated day lengths over the year.
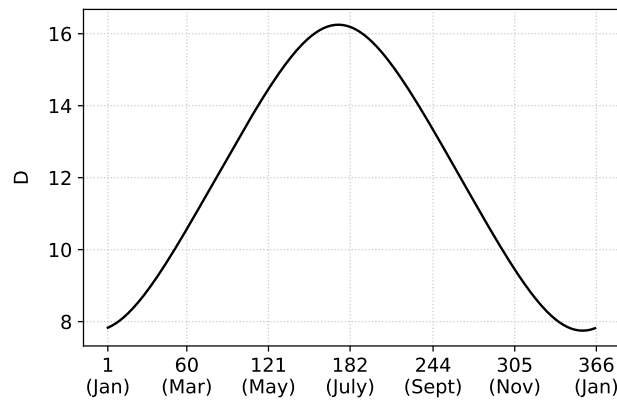
Figure 4.7: Daylength (D) at the latitude $50°6'$N.

4.4.8 Sub-models

Time component

Compartments   The model is based on 9 compartments (ordinary differential equations) that describe the populations and infection stages of mosquitoes and birds by means of a density-dependent approach (equations 4.2.1-4.3.5). The mosquito compartments include larvae (L_M), susceptible (S_M), exposed (E_M) and infectious mosquitoes (I_M). The bird compartments are susceptible (S_B), exposed (E_B), infectious (I_B), recovered (R_B) and dead birds (R_B). Recovered birds have always acquired immunity and the dead birds refer only to those that have succumbed to WNV.

The equations are taken from previous compartment models for Usutu virus and WNV [61, 63], but we introduced a species specific probability of the mosquitoes taking a blood meal on a bird and an additional probability of taking blood meals on a host bird when feeding on a bird. Both factors affect the virus transmission rates. We have also changed parameters that describe the characteristics of host birds and vector mosquitoes. All parameters of the differential equations are summarised in the tables 4.2, 4.3 and 4.4 and explained in the following.

Mosquito compartments

$$\frac{dL_M}{dt} = (b_L(T)\delta_M(D)N_M - m_M(T)L_M)\left(1 - \frac{L_M}{K_M}\right) - b_M(T)L_M \tag{4.2.1}$$

$$\frac{dS_M}{dt} = b_M(T)L_M - m_M(T)S_M - \lambda_{BM}(T,D)S_M \tag{4.2.2}$$

$$\frac{dE_M}{dt} = \lambda_{BM}(T,D)S_M - \gamma M(T)E_M - m_M(T)E_M \tag{4.2.3}$$

$$\frac{dI_M}{dt} = \gamma M(T)E_M - m_M(T)I_M \tag{4.2.4}$$

Bird compartments

$$\frac{dS_B}{dt} = \left(b_B - (b_B - m_B)\frac{N_B}{K_B}\right)N_B - \lambda_{MB}(T,D)S_B - m_B S_B \tag{4.3.1}$$

$$\frac{dE_B}{dt} = \lambda_{MB}(T,D)S_B - \gamma_B E_B - m_B E_B \tag{4.3.2}$$

$$\frac{dI_B}{dt} = \gamma_B E_B - \alpha_B I_B - m_B I_B \tag{4.3.3}$$

$$\frac{dR_B}{dt} = (1 - \nu_B)\alpha_B I_B - m_B R_B \tag{4.3.4}$$

$$\frac{dD_B}{dt} = \nu_B \alpha_B I_B \tag{4.3.5}$$

State variables

Average mortality rate of magpies   On average, Eurasian magpies get about 1.95 years old, most of them do not survive the first year [83]. This results in a mortality rate (m_B) of $0.5128\,\mathrm{y}^{-1}$ and $0.001404\,\mathrm{d}^{-1}$.

Rate infected-infectious in magpies   According to Oya et al. (2018) [25] who conducted an infection study on magpies with WNV lineage 1 - NY-99 (infection of 12 magpies) as well as with lineage 2 - SRB Novi-Sad/12 (infection of 11 magpies), positive serum samples can be

detected at 3 days post-infection with titres high enough to infect mosquitoes. Therefore, the rate infected-infectious, defined as reciprocal of the intrinsic incubation period (γ_B) is 0.333 d^{-1}.

Virus-induced mortality in magpies The death rate in the study from Oya et al. (2018) [25] was slightly lower for WNV lineage 1 (30 %) than for WNV lineage 2 (42.8 %). Since the infections in Germany were caused by WNV-2 so far, we have set the proportion of birds dying due to West Nile disease (ν_B) at 0.43.

Probability of infection in magpies The probability of the magpie being infected with WNV after contact with an infectious mosquito is not specifically known. Magpies having been inoculated with sera containing viraemic loads of 5×10^3 PFU have all developed symptoms of WNV disease [25]. Thus, the Eurasian magpie does not appear to have any resistance to infection.

Still the mosquitoes could not transmit a sufficiently high viral load during every blood meal. However, also in the previous model [61] the authors assumed that, in the case of the *Culex agg.*, every contact of an infected mosquito with a susceptible bird leads to virus transmission. For *Ae. j. japonicus*, a high transmission rate of 97 % was found in infection experiments on chickens [42]. Since there is no evidence that the transmission probabilities of infectious mosquitoes is species specific, we set the virus transmission rate from infectious mosquito to susceptible bird (p_M) to 100 % for both, *Ae. j. japonicus* and the *Culex aggregate*.

Removal rate of infectious magpies The infected birds in the experiments died on average 6.5 days after infection with WNV-2 [25]. This gives a time window in which they are contagious (high viraemia) of 3.5 days based on the assumption that they were infected from day 3, and a removal rate (α_B) of $0.29d^{-1}$.

Virus transmission from bird to mosquito For a mosquito to become infectious itself after an infectious blood meal, the virus must pass through the intestinal barrier, spread throughout the body and finally reach the salivary glands. A suitable measure for detecting a disseminated infection is the presence of the virus in the legs and wings [40].

In infection experiments with *Ae. j. japonicus* from a Swiss population conducted under representative European midsummer conditions [40], dissemination rates of 18.3 % (11 from 60 fed individuals) and 15.6 % (12 from 77 fed individuals) were determined for two sub-strains of WNV-1 (NY99 and FIN). Since infections with both is possible in Europe, we use the average of $p_{B_J} \approx 17 \%$ for *Ae. j. japonicus*.

Two further experiments [39, 42] with mosquitoes from populations from Maryland in the USA investigated WNV-dissemination rates of both *Ae. j. japonicus* and *Cx. pipiens sl.*. The biotype of *Cx. pipiens* is not documented. Both experiments were carried out in the same laboratory, at constant temperatures of 26°C, and in one of them the effects of two different virus titres on both species were also investigated. For *Ae. j. japonicus* dissemination rates of 65.7 % on average were found. For *Cx. pipiens sl.* they were 2.8 times lower with 23.3 % on average.

Since the Swiss experiment was conducted under more realistic conditions for Central Europe and since, according to [60], typical dissemination rates are between 2 % and 24 %, we use the result from this study for *Ae. j. japonicus*. p_{B_C} is set at 17/2.8= 6 % according to the studies from the USA.

Degree of ornithophilia   The previous model [61] was based on the assumption that species from the *Culex aggregate* feed from birds exclusively. This theory is supported by several blood meal analyses [84–86]. However, there are also numerous deviating observations. Large-scale studies in Germany [87], the USA [64], and Portugal [88] have found an average of 33 %, 63 % and 73 % of the blood-sucked females belonging to the *Culex* genus to have fed on birds. *Culex pipiens* with the two biotypes *pipiens* and *molestus* as well as *Cx. torrentium* were among them, each with different proportions. Furthermore, blood meal analyses from mosquitoes trapped in the zoo of Zürich (Switzerland) [89] are worth mentioning, as mosquitoes in this habitat should have a wide range of potential host species available. In this zoo, the proportion of *Culex* mosquitoes that fed on birds was 96 %. The specimens belonged to the species *Cx. pipiens sl.* (biotype not specified) and *Cx. torrentium*.

Possible reasons for varying observations in different regions are the composition and densities of potential host species and their defence behaviour [90]. Furthermore, the mosquito trapping method influences the results of blood meal analyses [87]. Since no clear statement can be made on the degree of ornithophilia of *Cx. pipiens*, we perform scenario analyses with $P_C = 96\%$ [89], 33 % [87] and 66 %. The last value is the average of all aforementioned studies.

Aedes j. japonicus is described in the literature as mammalophilic [84]. A study in the zoo in Zürich [89] was the only one where bird blood was detected in individuals from the field. In this study, the percentage of avian blood fed to all mosquito females that had ingested blood was 15.5 %. In addition, mixed blood meals were detected in nearly 2 % so that we assume an overall degree of ornithophilia (P_J) of 16.5 %.

Further evidence that *Ae. j. japonicus* females accept birds for their blood meals was provided from the laboratory [91]. In addition, WNV has been detected in individuals captured in the field [91, 92], suggesting that the mosquitoes may also actively seek out birds to some extent.

Table 4.2: State variables

Term	Description	Value
m_B	Average mortality rate of magpies	$0.001404\,\mathrm{d}^{-1}$
γ_B	Rate infected–infectious magpies	$0.333\,\mathrm{d}^{-1}$
ν_B	Portion of magpies dying due to WNV infection	0.43
p_M	Transmission efficiency from infectious mosquito to bird	1.0
α_B	Removal rate of magpies due to WNV infection	$0.28\,\mathrm{d}^{-1}$
p_{B_J}	Transmission efficiency from infectious bird to *Ae. j. japonicus*	0.17
p_{B_C}	Transmission efficiency from infectious bird to *Culex agg.*	0.06
P_J	Proportion of *Ae. j. japonicus* blood meals taken on birds	0.18
P_C	Proportion of *Culex agg.* blood meals taken on birds	0.33, 0.66, 0.96

Temperature-dependent parameters

Mosquito biting, development and death rates   The biting rate $k(T)$ indicates how often mosquitoes take blood meals and depends on the temperature-dependent protein digestion and the resulting duration of the gonotrophic cycle [61]. The larval hatching rate, the mosquito birth rate, and the virus transmission rate from mosquitoes to birds are derived from the biting rate (Fig. 4.8 a).

For the biting rate of the *Culex agg.* we use the function from the previous model [61] (Tab. 4.3). The same function was applied to *Ae. j. japonicus* because we found no evidence in the literature that the rate varies between different mosquito species.

Concerning the temperature-dependent development and mortality of *Ae. j. japonicus* in the imaginal and pre-imaginal stage, results from several laboratory studies exist [93–95]. However, we conclude from the different survival and development rates that the results of rearing experiments with possibly different rearing conditions cannot be directly compared. Nevertheless, for *Ae. j. japonicus* larvae, the mortality rate was found to be lowest at 20°C [94], as is provided in the original model for the *Culex agg.* [61]. Based on the above considerations, we applied the equations from the original model for both mosquito species (Tab. 4.3, Fig. 4.8).
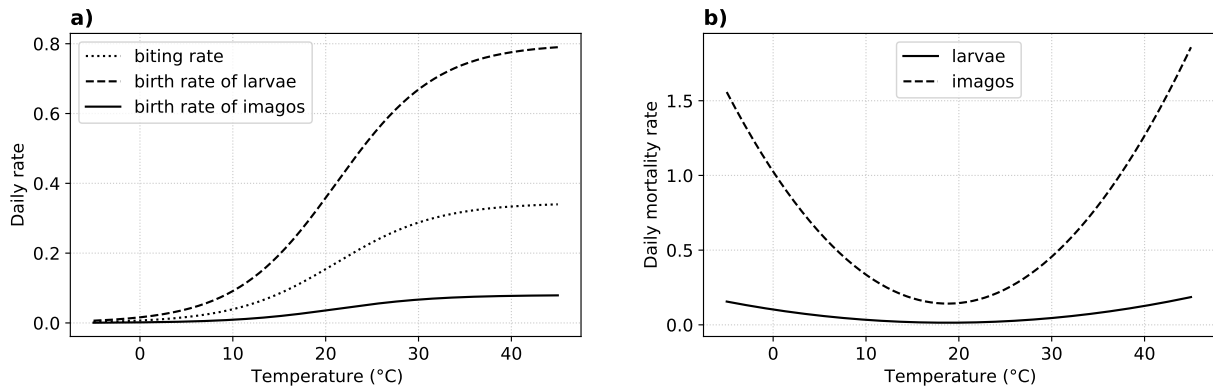
Figure 4.8: a) Biting rate and birth rates of mosquito larvae and imagoes. b) Mortality rates of larvae and imagoes. The functions apply to both *Aedes japonicus japonicus* and *Culex pipiens sl.*.

Virus transmission rates   The transmission rate from an infectious mosquito to a susceptible bird corresponds to the product of the species-specific bird biting rate $Pk(T)$, the proportion of bird bites on host birds, and the transmission efficiency p_M (Fig. 4.9, Tab. 4.3). The transmission efficiency is 1 for both species here.

The transmission rate from an infectious bird to a susceptible mosquito ($\beta_B(T)$) is the product of the bird biting rate, the proportion of bird bites on host birds, and the transmission efficiency p_B which is specific to the mosquito species (Fig. 4.9, Tab. 4.3).

To what extent *Ae. j. japonicus* and the species of the *Culex* complex visit the WNV host bird species compared to other bird species is not specifically known for central Europe. It is likely that bird species whose habitats overlap with those of the mosquitoes will be preferred, and that a high bird species diversity reduces the likelihood of a vector mosquito encountering a host bird.

We made model applications with the assumption that both, the species of the *Culex agg.* and *Ae. j. japonicus*, do not prefer any bird species for their blood meals. Since in Germany the magpie accounts for about 5.7 % of the total bird population [69, p. 59 and p. 397], we integrated that factor in the transmission rates and found that, with this setting, transmission cycles cannot occur in any scenario. Only when at least 50 % of the avian blood meals are taken on the host bird, epizootics can be triggered in the warmest region of Germany (R 3). This is why we work with a 75 % preference factor for magpies over other bird species in the model applications presented here.

Rate infected-infectious among the mosquitoes   The rate infected-infectious among the mosquitoes (γ_M) is the reciprocal of the extrinsic incubation period (EIP). The EIP is temperature-dependent [41, 63], but there is only one study that investigated the influence of a wider range
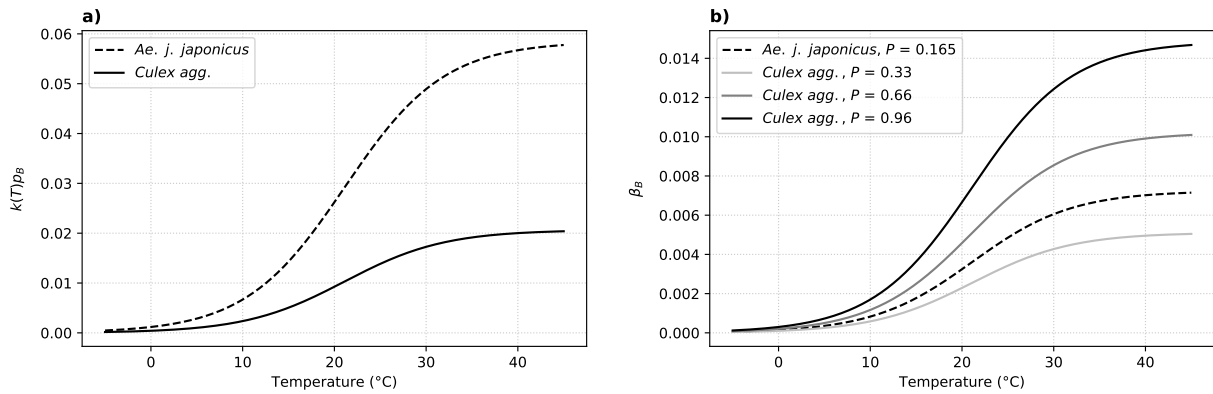
Figure 4.9: a) Potential virus transmission rate from bird to mosquito, defined as the biting rate multiplied by the probability of transmission from an infectious bird to the mosquito and b) transmission rates taking into account the degree of ornithophilia (P_M) and the proportion of bird bites taken on the host birds.

of temperatures on the EIP for a *Culex* species (*Cx. tarsalis*) [96]. There is evidence that the EIP for *Ae. j. japonicus* is shorter than for species of the *Culex* complex. In a study conducted at constant temperatures of 26°C, viraemia could be detected 3 days post-infection in *Ae. j. japonicus* compared to 11 to 12 days in *Cx. pipiens sl.*. However, titres were still low on the first days with evidence of disseminated infection in *Ae. j. japonicus* and no re-infection was attempted in birds with these low titres.

Due to the scarce data on temperature-dependent EIP in *Ae. j. japonicus* and other species of the *Culex agg.*, we apply the same function for both model applications as in the previous models [61, 63], which is derived from the study mentioned above [96] (Fig. 4.10).
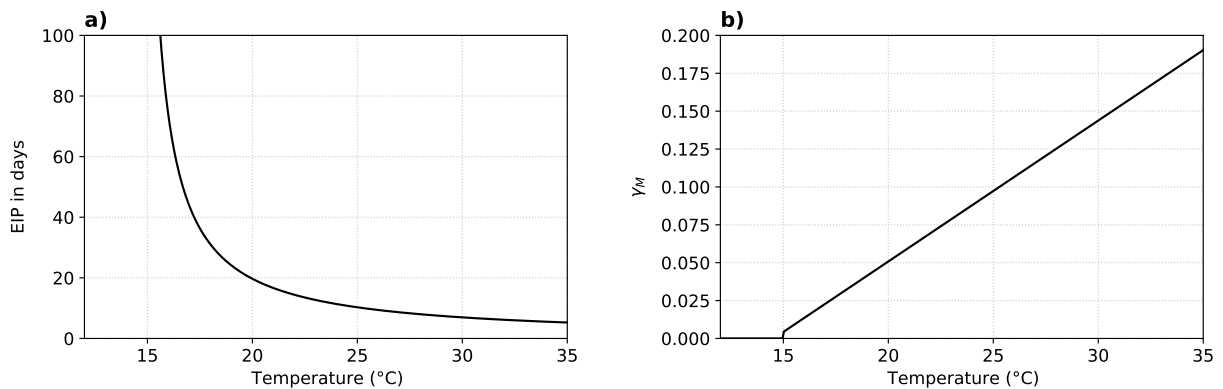
Figure 4.10: a) Extrinsic incubation period and b) rate infected-infectious among *Aedes japonicus japonicus* and the *Culex*

Daylength and season dependent parameters

Table 4.3: Temperature-dependent parameters

Parameter description	Function
Biting rate	$k(T) = \dfrac{0.344}{1 + 1.231e^{-0.184T - 20}}$
Larva birth rate	$b_L(T) = 2.325k(T)$
Mosquito birth rate	$b_M(T) = 0.1b_L(T)$
Larva mortality rate	$m_L(T) = 0.0025T^{2-0.094T+1.0257}$
Mosquito mortality rate	$m_M(T) = 0.1m_L(T)$
Transmission rate I_M to S_B	$\beta_M(T) = 0.75Pk(T)p_M$
Transmission rate I_B to S_M	$\beta_B(T) = 0.75Pk(T)p_B$
Rate infected-infectious mosquitoes	$\gamma_M(T) = \dfrac{1}{0.0093T - 0.1352}$ for $T > 15$; else: 0

Fraction of active mosquitoes (δ_M) The proportion of mosquitoes that are active (not diapausing) depending on the daylength, was taken from Rubel et al. (2008) [63] for the *Culex agg.* (Tab. 4.4). It has often been reported from temperate regions that the imagoes of *Ae. j. japonicus* occur much longer in the year than those of other mosquito species [97]. Most findings are from the period between May and October, but there are also some already from March and until December [48, 97, 98]. Based on these references we have adjusted the activity function for *Ae. j. japonicus*. (Fig. 4.11, Tab. 4.4).
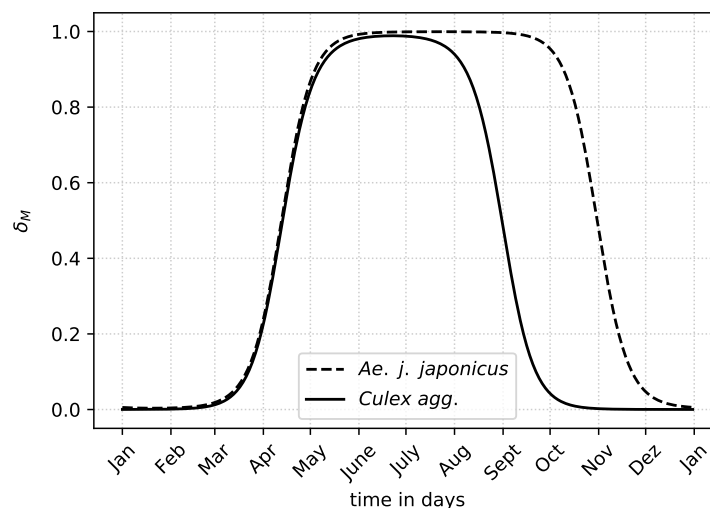
Figure 4.11: Fraction of active, not diapausing mosquitoes.

Bird birth rate (b_B)   Breeding pairs of magpies get about 1.4 fledglings (average value from several studies [79]). Since 14 % of the magpies do not breed, one can conclude that every territory consists of about 2.28 birds that get on average $1.4/2.28 = 0.614$ fledglings per year each.

According to [61] we fitted a gamma distribution to observation data of breeding success [99] (Fig. 4.12). The data are given as a percentage of the young birds, which left the nests in observation periods of 10 days, from the total annual observed breeding success. We fitted the following parameters for the gamma distribution: $\alpha = 4.43$, $\beta = 7.67$ and location$= 120$ (Table 4.4). The location parameter corresponds to the transformed Julian calendar day. We multiplied the gamma function with the calculated average of fledglings per magpie of 0.614 to obtain the mean birth rate of every single bird in the course of the year.
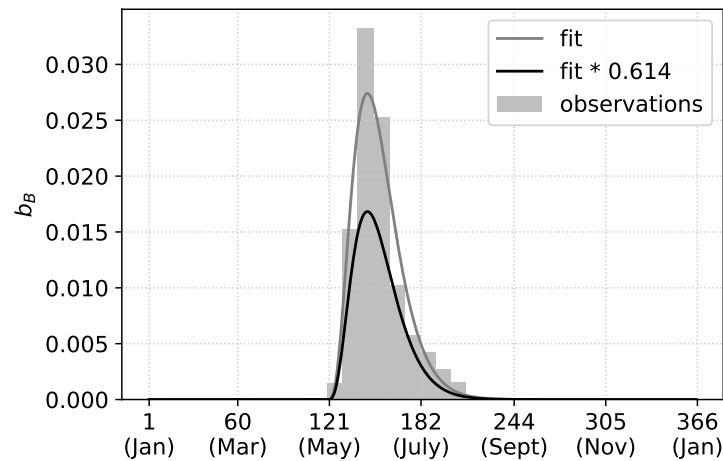
Figure 4.12: Birth rate of Eurasian magpies (*Pica pica*) in central Europe as function of the day of the year.

Force of infection   Infection occurs upon mixing a fraction of infectious and susceptible mosquitoes or birds, respectively. The number of susceptible mosquitoes exposed on a simulation day (λ_{BM}) depends on the proportion of active mosquitoes (δ_M), the transmission rate from infectious bird to susceptible mosquito (β_B), and the density of infectious birds defined as the fraction of infectious birds to its maximum density (Eq. 4.4.1).

The number of susceptible birds exposed to WNV also depends on the fraction of active mosquitoes, on the transmission rate from infectious mosquito to susceptible bird (β_M), the density of infectious mosquitoes defined as fraction of the number of infectious mosquitoes to

Table 4.4: Daylength and season dependent parameters

Parameter description	Function
Fraction active *Ae. j. japonicus*	$\delta_J(D) = 1 - \dfrac{1}{1 + 40,000e^{1.559(D(d-30)-18.177)}}$
Fraction active *Culex agg.*	$\delta_C(D) = 1 - \dfrac{1}{1 + 1,775.7e^{1.559(D(d))-18.177}}$
Birth rate magpies	$b_B(x) = 0.614\dfrac{(x/\beta)^{\alpha-1}e^{-(x/\beta)}}{\beta\Gamma(\alpha)}, x > 0, \alpha = 4.43, \beta = 7.67$
Transformed Julian calendar day	$x = d - 120$

the maximum density (I_M/K_M), and furthermore on the average ratio of vector mosquitoes to host birds (K_M/K_B) (Eq. 4.4.2).

$$\lambda_{BM}(D,T) = \delta_M(D)\beta_B(T)\frac{I_B}{K_B} \tag{4.4.1}$$

$$\lambda_{MB}(D,T) = \delta_M(D)\beta_M(T)\frac{K_M}{K_B}\frac{I_M}{K_M} \tag{4.4.2}$$

Solving the differential equations and virus introduction The differential equations were solved with the Euler method. To trigger West Nile virus infections in the study regions during a model application, we introduce infectious birds into the system 3 times a year: 2 at the beginning of March (after the arrival of migratory birds), 2 at the end of July (end of the main chick hatching period) and 4 at the beginning of October, as some bird species migrate intercontinental in autumn.

Spatial component

There are 2 types of functions in the spatial model component: firstly, those that relate to the individual behaviour of the mosquitoes and secondly those that control the mosquito population in the region as a whole.

Individual behaviour of the mosquitoes

Super-agents   In order to achieve acceptable runtimes, several of the female mosquito ima-
goes with the same infection status are bundled together to form a super-mosquito. One super-
mosquito can comprise 10, 100 or 1000 individuals, depending on the setting, and it is equipped
with the appropriate characteristics: the study region, position within the grid, stage of infection,
energy-level and information on whether an attempt has been made to leave the region and if so,
in which direction.

Flight behaviour   With each simulation step, the super-mosquitoes can move on the grid
map with 100 m $\times$ 100 m resolution in any direction within the Moore neighbourhood. The
distance they travel is controlled by a flight motivation resulting from the habitat suitability of the
current location. The motivations and resulting distances were defined as follows:

Active dispersal rates of female mosquitoes are generally dependent on the density and distri-
bution of their preferred blood hosts, the availability of breeding habitats, terrain characteristics
and weather [100, 101]. Regarding terrain characteristics, vegetation and land use play a particu-
larly important role. They can represent natural barriers, ecological corridors and stepping stones
for dispersal. Landscapes that support the spread of *Ae. j. japonicus* are mosaics of smaller de-
ciduous forests (less than 500 ha), vineyards and areas with private houses and gardens. Barriers,
however, are e.g. large forest areas and intensively cultivated fields [102–104].

Based on the insights described above, we have introduced a flight motivation in the flight
simulator which is landscape dependent. We differentiate between low, medium and high mo-
tivations, which are assigned to the mosquitoes when they are in cells with values ≥ 0.8 to 1.0,
≥ 0.3 to 0.8 and 0 to < 0.3. According to the model of Kerkow et al. (2019) [70] whose output
we use as a basis for the "mosquito worlds" in the flight simulator, the category with the highest
habitat quality (1.0 to ≥ 0.8) includes discontinuous urban fabrics, green urban areas, cemeteries,
gardens, zoological gardens and deciduous forests. The middle one (0.8 to ≥ 0.3) covers most
notably mixed forests, woodland-shrub transition zones, fruit and berry plantations, and the last
one (< 0.3 to 0) mainly landscapes with an open character such as farmland, pastures and airports,
but also continuous urban fabrics and coniferous forests.

For *Ae. j. japonicus* we are not aware of any published results of mark-recapture experiments
that would help to estimate the flight distances in certain landscapes and under certain weather
conditions. There is evidence that individuals in a perfect habitat do not fly long distances and
hardly spread out, which may explain the findings that larger contiguous forest areas sometimes
act more as barriers than as corridors. For a location in Belgium (a second-hand tire company
with a neighbouring small woodland) no evidence of its dispersion from there could be found

even after 6 years, although there are suitable stepping stones, a settlement in direct connection as well as further woodlands and tree rows at distances of only a few hundred metres linear distance [105]. Also, species of the genus *Aedes* and especially the urban-domestic, container-breeding species are known to be rather weak flyers [101] and no behaviour like that of long-distance fliers was documented for *Ae. j. japonicus*, i.e. ascending high into the air immediately after adult emergence, facilitating far wind drifts [106].

An approximate indication of flight distances is given by a documentation of the spread of *Ae. j. japonicus* in West Germany (North Rhine-Westphalia) [75]. There, an average dispersal of about 10 km to 20 km per year was recorded between 2012 and 2015, with some exceptions of up to 50 km. As it is known that *Ae. j. japonicus* also spreads passively to a large extent, especially along roads [105], we suggest that the exceptional dispersal rates exceeding 2 km/year are due to this cause. Assuming that the annual main activity and thus the dispersal period is 200 days/year (from mid-March to mid-October), the maximum flight distances would range from 500 m to 1 km/day.

Evaluations of mark-recapture experiments of various mosquito species show that most individuals remain at the site and the probability of recapture decreases with increasing distance [101]. To the summarised data on *Ae. albopictus*, which is also described as a weak flyer like *Ae. j. japonicus* and which has similar habitat requirements, we have fitted a normal distribution (Fig. 4.13a) to generate probabilities for the flight ranges of *Ae. j. japonicus* agents with medium flight motivation. The data was divided by 100 before the fitting to convert the meters to map units. The distribution has the mean value at zero and a standard deviation (σ) of 2.4. For mosquitoes with low flight motivation, we have reduced the standard deviation of the fitted normal distribution to 0.6 so that they rarely fly further than 100 m or 200 m and the probability of staying in the cell is relatively high (Fig. 4.13b).

In order to determine the flight direction and next location of a low and medium motivated mosquito on the map, its flight range is randomly divided into two segments. They represent the horizontal and vertical direction of movement on the grid. Whether the vertical direction is towards the south or north and the horizontal direction towards the west or east is determined by the algebraic sign of the randomly generated flight distance and by means of another random generator.

We assume that the mosquitoes fly longer distances in unsuitable habitats as they are not able to satisfy all their needs (availability of breeding habitats, host animals or shelter provided by vegetation). Therefore, we implemented a random generator for mosquitoes in these habitats, which generates equally distributed flight distances between 0 and 3 to 4 map units for the
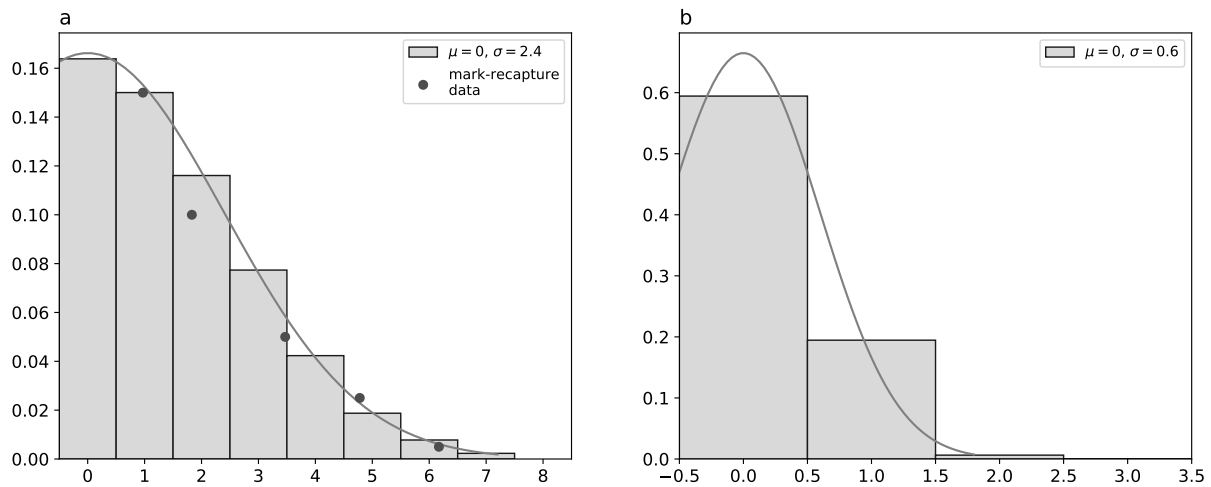
Figure 4.13: Distribution curves used to generate random flight distances for a) moderately motivated and b) low motivated mosquitoes.

horizontal axis and the vertical axis respectively. This results in a maximum flight distance of 566 m to 800 m per day, depending on whether the mosquito flies the direct route.

Various studies in wind tunnels and in the field show that mosquitoes are more likely to fly upwind than downwind [107–109]. This is probably due to the appetising flights in which they follow smells that are transported by the wind [101]. At wind speeds of 3 km/h and above, however, many species stop flying and seek shelter on the ground or in the vegetation [106].

As stated above, the poor mosquito habitats are mainly open landscapes offering limited protection, so we assume that the influence of the wind is particularly noticeable in these. Using data from the surrounding weather stations of the study regions, we analysed the main wind directions and found that for each region, movement in a westerly direction on the horizontal axis and in a southerly direction on the vertical axis would be more likely (Fig. 4.14). Hence, we let the mosquitoes fly up to 400 m towards west and south and up to 300 m towards north or east. This results in a 1.3 times higher probability of a mosquito flying in the direction with the stronger wind conditions.

Boundary effects   We have implemented two options for the area boundaries of the study regions. In "open" mode, the mosquitoes can leave the region and the loss is compensated by births of mosquitoes with the same infection status. This mode merely prevents a potential mass accumulation at the edge of the study region. In "closed" mode, the mosquitoes cannot leave the region. An emigration attempt is registered and the cardinal point is saved on the first time. However, the flight is not executed and the super-mosquito remains in its cell.
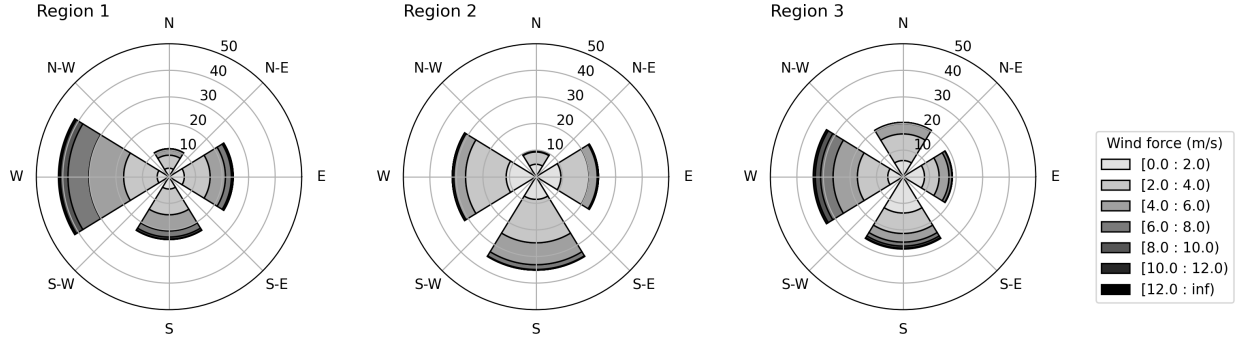
Figure 4.14: Wind roses showing the multi-annual distribution averages of wind strengths and directions for the 3 study sites within the period 2011-2019.

The distribution pattern hardly differs between the applications of both options (we could not observe any mass accumulation of mosquitoes at the borders in closed mode). However, the calculation time is significantly longer in the open mode, so we will only apply the closed mode in this work.

Energy   The mosquitoes in the flight simulator have an energy level that causes them to die during long stays in unsuitable habitats or after covering large distances. This makes large areas with unfavourable conditions to dispersion barriers. It is known that long distance flights are highly energy consuming for mosquitoes [101], but we have no information about the distance covered by *Ae. j. japonicus* under certain habitat conditions. We thus adapted the energy control in a way that a significant reduction of the population can occur in region 1, but the population remains stable in regions 2 and 3. This is consistent with observations made in systematic monitorings [44, 75].

For the energy level of a mosquito, which can take on values between 1 and 100, we use the following balance equation:

$$\frac{dE}{dt} = \delta_{\text{prev}}E - \delta_{\text{const}} - \delta_{\text{flight}}F + bQ \tag{4.5}$$

where δ_{prev} is the inverse time unit, E is the energy on the previous time unit, δ_{const} is a constant energy loss per time unit, δ_{flight} is the energy loss per flight distance and time unit, F is the Euclidian flight distance between the start and destination cells (the cells have a resolution of $100 \times 100\,\text{m}^2$, so the distance is given in hectometres), b is a boosting factor per time unit, and Q is the quality value of the new cell (the quality values are between 0 and 1 and we handle them equivalent to energy units per time unit). For the simulations, we set $\delta_{\text{prev}} = 1$ per time unit,

$\delta_{const} = 4$ EU (energy units) per time unit, $\delta_{flight} = 8$ EU per time unit and flight distance, and $b = 50$ EU per time unit.

Control of all mosquitoes   To apply certain processes to all super-mosquitoes in the simulation, we have drawn up two lists at a higher hierarchical level in the program. They contain the infectious mosquitoes and those with other infection status, with all their characteristics (the exposed and the susceptible mosquitoes are aggregated in the spatial component). On this level, we also count the number of mosquitoes that have tried to leave the region and whether it was the first time.

Deaths   If the temporal model component reduces the number of mosquitoes compared to the previous day (this happens almost every day in autumn) and if, as a result, the number of mosquitoes is lower than in the spatial component, then the mosquitoes are also removed from the spatial component. Depending on whether it is an infectious mosquito or not, a random one is removed from the corresponding list. Mosquitoes that have died due to a loss of energy are already included in the comparison.

Births   The birth function is used on the one hand when the SEIR model calculates an increase in mosquitoes compared to the last day and on the other hand when mosquitoes have emigrated in the open model mode and this loss is to be compensated. By births the super-mosquitoes appear at random locations in the study area, preferably in patches with good habitat qualities. Details of this implementation are identical to the initial distribution of mosquitoes on the first day of the simulation (see Chapter 4.4.6).

4.5   Results

4.5.1   Linked model and application to Aedes japonicus japonicus

The results of the spatial distributions of the *Ae. j. japonicus* populations at the time when most infectious mosquitoes are present show that the individuals accumulate strongly in cells with very good habitat qualities (values from 0.8 to 1) (Fig. 4.15). The majority of these cells contain deciduous forests and human settlements in all 3 study regions (Fig. 4.1, 4.3).

In region 1, very poor and very good habitats are particularly strongly separated from each other (Fig. 4.3 a). This enables to assess the spatial extent at which a dispersion barrier is effect-ive. We found that the energy and flight distance settings resulted in the mosquitoes moving a

maximum of about 2 km away from the very good habitats. Already at a distance of 1 km, the mosquito density decreases rapidly. As a result, the area with the poor conditions in the centre was not a strong dispersion barrier, but an area in the north-east of the region remained completely mosquito-free throughout the simulation (Video S1, Fig. 4.15 a).

Also in region 2, many mosquitoes are able to cross the area with poor habitat characteristics, which extends from the north-west to the centre and is mainly characterised by arable land (video S2, Fig. 4.15 c). Small towns and villages between the crop lands serve as stepping stones (Fig. 4.1 b).

Table 4.5: Emigration attempts and energy-related deaths of super-mosquitoes (1 super-mosquito refers to 10 individuals) in the application of the linked model for the year 2018

	First emigration attempts						Total attempts	Energy-related deaths	
	North	East	South	West	Others	Sum		$S_M + E_M$	I_M
Region 1	12,167	6,165	34,922	34,542	14	87,810	111,701,473	228,685	1
Region 2	43,028	44,251	42,274	12,960	11	142,524	217,099,996	53,621	0
Region 3	22,923	49,596	50,841	31,226	7	154,593	321,748,672	134,199	0

The number of super-mosquitoes being eliminated from the simulation during the entire simulation period due to low energy levels was about 228,700, 53,600 and 134,200 for regions 1, 2 and 3, respectively (Tab. 4.5). One super-mosquito corresponds to 10 individuals and the simulation period refers to the year 2018. The reduction in total maximum mosquito population from applications of the linked model in 2018 compared to the SEIR model in 2018 was 9.82 % in Region 1, 1.1 % in Region 2, and 0.05 % in Region 3 (Table 4.6, Fig. 4.16, 4.17, 4.18).

In region 1, repeated use of model applications resulted in a higher risk of infected super-mosquitoes being prematurely removed from the simulation than in the regions 2 and 3. This is due to the large proportion of unfavourable habitats in region 1. The infection period in the coupled model lasts 84 days in region 1 (start on day 227), 136 days in region 2 (start on day 219) and 149 days in region 3 (start on day 216) (Fig. 4.16, 4.17, 4.18).

The number of total emigration attempts is almost three times higher in region 3 and almost twice as high in region 2 than in region 1. However, if only the first emigration attempts are considered, the differences are smaller (Tab. 4.5, Fig. 4.19). In region 1, approximately the same number of super-mosquitoes emigrate towards south and west during the entire simulation. On the one hand, these are the main wind directions in all study regions (Fig. 4.14) and, on the other hand, more areas with high occurrence probabilities have intersections with the borders in the west and south of region 1.

In region 2, about the same number of mosquitoes made the first attempt to emigrate towards the north, east and south. Much less good and very good mosquito habitats border the eastern edge of the region, and only a third of the super-mosquitoes tried to leave the region for the first time in this direction. In region 3, there were about twice as many first attempts to emigrate towards the east and south as towards the north or west. Also here, the result is related to the very good mosquito habitats located along the edge of the region and not to the main wind directions.

4.5.2 SEIR model applications

Applications of the SEIR model without spatial coupling and for the period 2018–2020 show that *Ae. j. japonicus* can build up slightly higher maximum population sizes compared to the *Culex agg.* (Fig. 4.20 a, 4.21 a, 4.22 a). Nevertheless, vectorial capacityas measured by the maximum number of infectious mosquitoes is 56 times higher in the *Culex* species aggregate with a degree of ornithophilia of 96 % on average (based on all 3 study regions) compared to *Ae. j. japonicus* with an ornithophilia of 16.5 % (Fig. 4.20 b, 4.21 b, 4.22 b, Tab. 4.6).

Table 4.6: Comparison of the maximum number of all and infected mosquitoes and birds

		Culex agg.			*Ae. j. japonicus* ($P_J = 0.165$)	
		$P_C = 0.33$	$P_C = 0.66$	$P_C = 0.96$	SEIR	Coupled model
Region 1	N_M	9,489,866.00	9,489,866.00	9,489,866.00	10,429,570.00	9,405,828.00
Region 1	I_M	28.02	536.66	4418.19	89.71	47.77
Region 1	I_B	2.92	30.06	248.22	5.76	4.40
Region 2	N_M	17,389,130.00	17,389,130.00	17,389,130.00	19,046,930.00	18,852,660.00
Region 2	I_M	97.15	2845.21	27830.71	372.22	362.76
Region 2	I_B	4.05	119.82	887.20	18.42	17.98
Region 3	N_M	24,651,690.00	24,651,690.00	24,651,690.00	26,893,620.00	26,881,040.00
Region 3	I_M	701.03	46660.80	229192.96	5241.68	5234.14
Region 3	I_B	22.19	1033.35	2511.91	225.22	224.94

With an ornithophilia of 66 % in the *Culex agg.*, the number of infectious mosquitoes is on average 7.6 times higher than in *Ae. j. japonicus*. An ornithophilia of 33 % in the *Culex agg.*, however, does not result in the emergence of an epizootic. The scenario with *Ae. j. japonicus* produces nearly 5 times more infectious mosquitoes in comparison.

A similar picture emerges when comparing the maximum number of infectious birds (Fig. 4.20 d, 4.21 d, 4.22 d, Tab. 4.6). Especially in region 3, the scenario with the *Culex agg.* acting as vector mosquitoes combined with a degree of ornithophilia of 96 % leads to the situation

that almost half of the magpies acquire immunity. As a result, only 11 % more infectious birds develop in this region despite the favourable temperature conditions and the high mosquito density compared to the scenario with *Ae. j. japonicus* acting as a vector. By contrast, in region 2 the difference was 48 %.

4.5.3   Runtime

The application of the coupled model to the year 2018 on a machine with an Intel(R) Core(TM) i7-4510U processor with 4 CPU lasts 9 min, 20 min and 28 min for the 3 study regions, respectively, and a super-mosquito factor of 100. Without saving the arrays for each simulation day which serves to create the flight videos, the runtime is reduced by about 3 min. With a super-mosquito factor of 1.000, the runtime can be reduced even more to a maximum of 3 min. However, with a factor of 10, the runtime is 6 h 13 min for region 1, 1 d 1 h 47 min for region 2 and 1 d 6h 22 min for region 3.

4.6   Discussion

We introduce the first compartment model for West Nile virus, which also simulates the abundance and movements of a vector mosquito species in a spatial extension and thus helps to estimate where virus hotspots could be located and towards which direction the virus is likely to be carried from a region by active flights of the mosquitoes. Both parts of the model, the spatial component (flight simulator/ ABM) and the temporal component (SEIR model), are exploratory. This means that the results are not yet validated. We have reviewed the literature and based on this, we implemented the agent-based flight simulator. Additionally, we adapted the SEIR model to the conditions in Central Europe and a realistic size of the mosquito population. Originally, the SEIR model was implemented for Montreal and validated with the number of detected dead sentinel birds [61].

4.6.1   Temporal model component

Validation of the results of our exploratory SEIR model applications is not possible because (i) the evidence of WNV-RNA in German wild bird populations is still very low, (ii) it is uncertain whether the Eurasian magpie is or will be a major vector in Central Europe, (iii) there are very likely several bird and mosquito species that act as efficient hosts and vectors, so the model should be extended to deal with this, and (iv) there is still a lack of clarity about multiple factors that
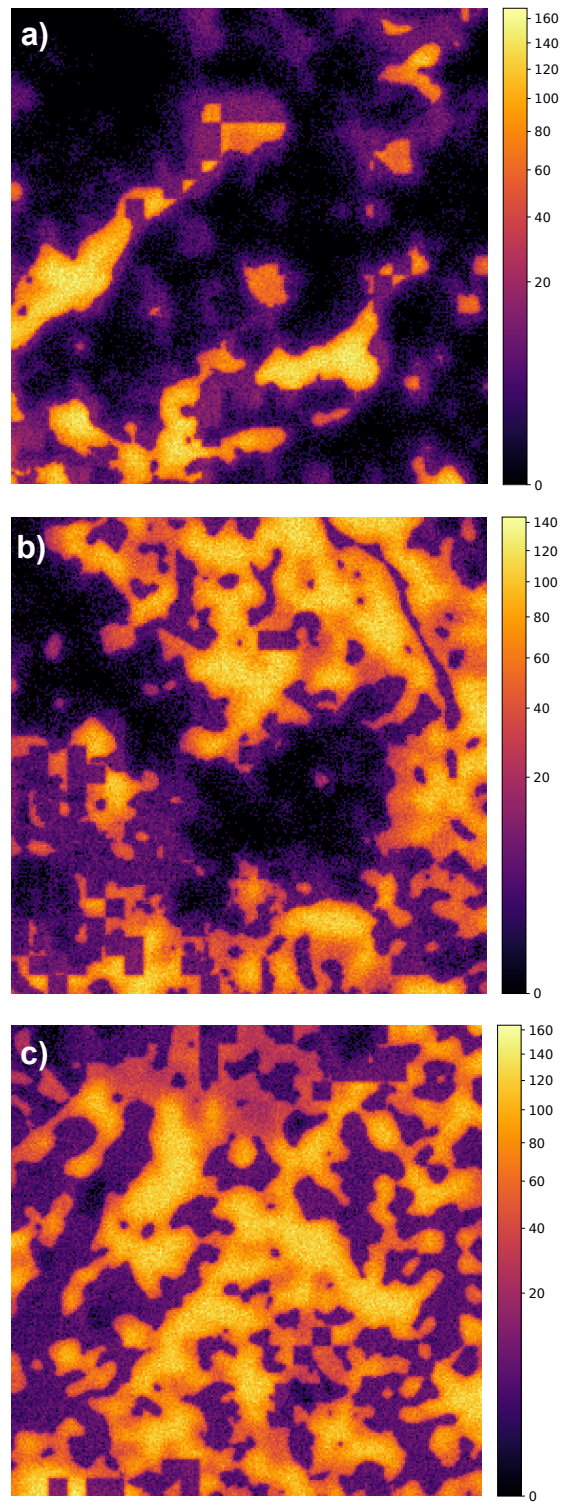
Figure 4.15: Distribution of female, non-diapausing mosquitoes in all stages of infection in study region 1 (a), 2 (b) and 3 (c); All sub-figures refer to the day with the highest number of infectious mosquitoes and are derived from model application for the year 2018.
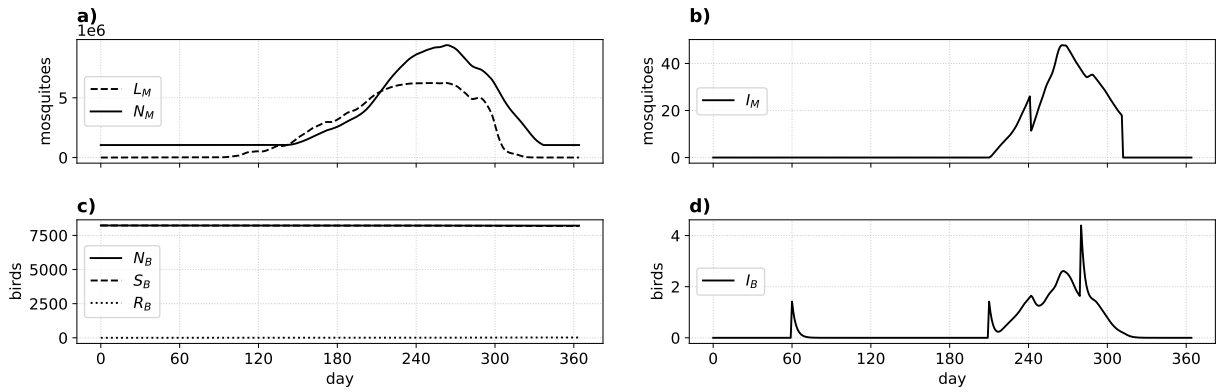
Figure 4.16: Results of the coupled model for the year 2018 and region 1 with *Aedes japonicus japonicus* as vector mosquito and the Eurasian magpie *Pica pica* as host bird.
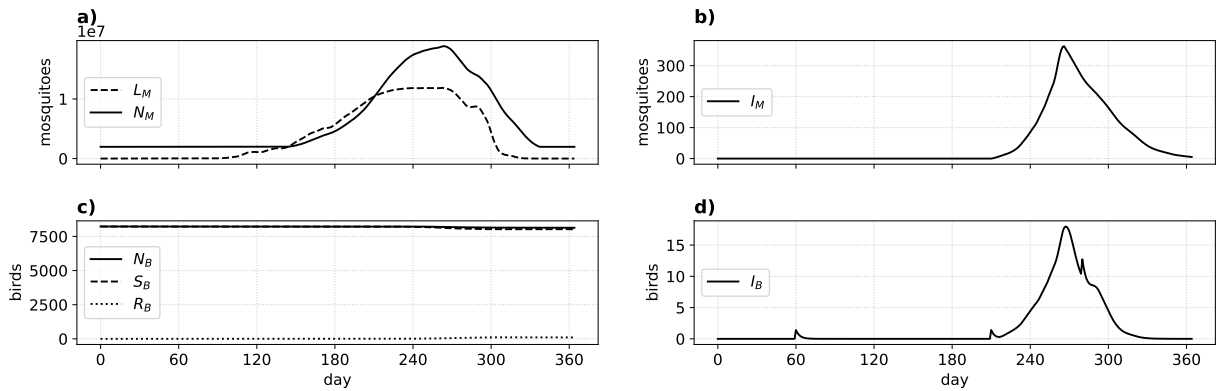
Figure 4.17: Results of the coupled model for the year 2018 and region 2 with *Aedes japonicus japonicus* as vector mosquito and the Eurasian magpie *Pica pica* as host bird.
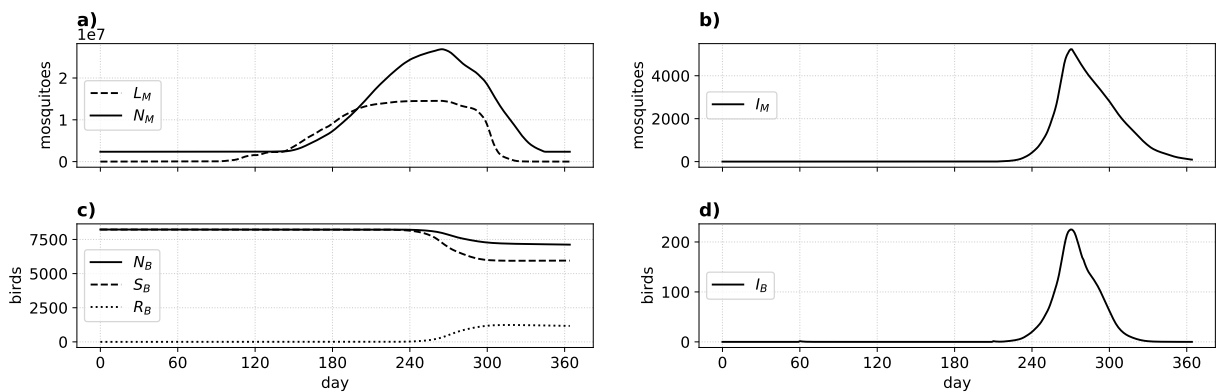
Figure 4.18: Results of the coupled model for the year 2018 and region 3 with *Aedes japonicus japonicus* as vector mosquito and the Eurasian magpie *Pica pica* as host bird.

a) Region 1

b) Region 2

c) Region 3
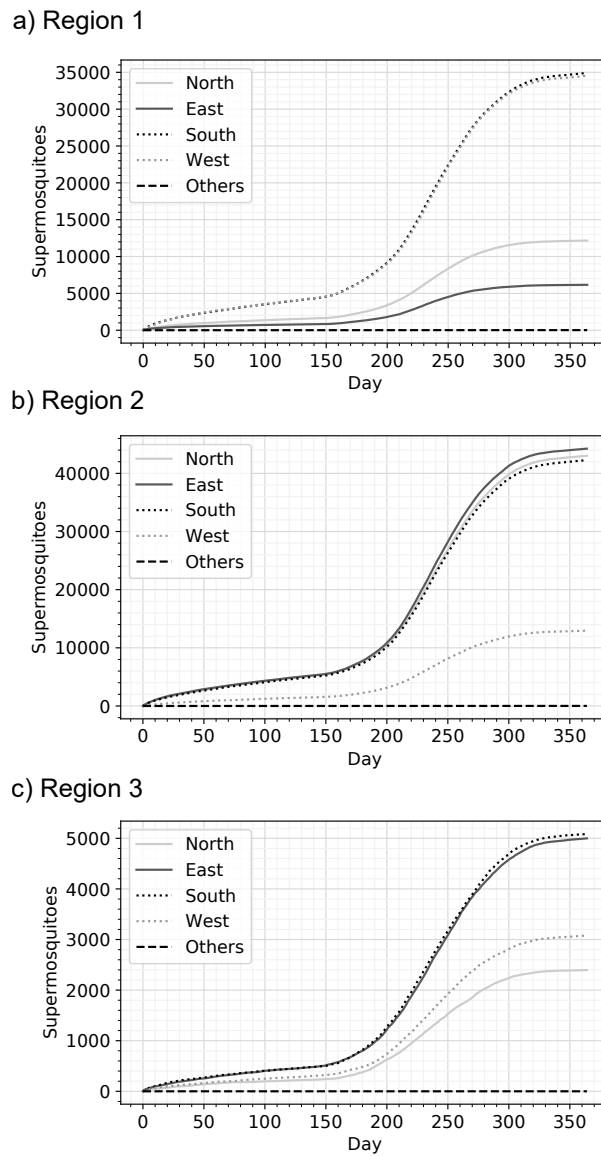
Figure 4.19: Daily cumulative number of mosquitoes that attempted to emigrate to each of the cardinal points for the first time during the simulation period. The term "Others" stands for the options north-east, south-east, south-west and north-west.
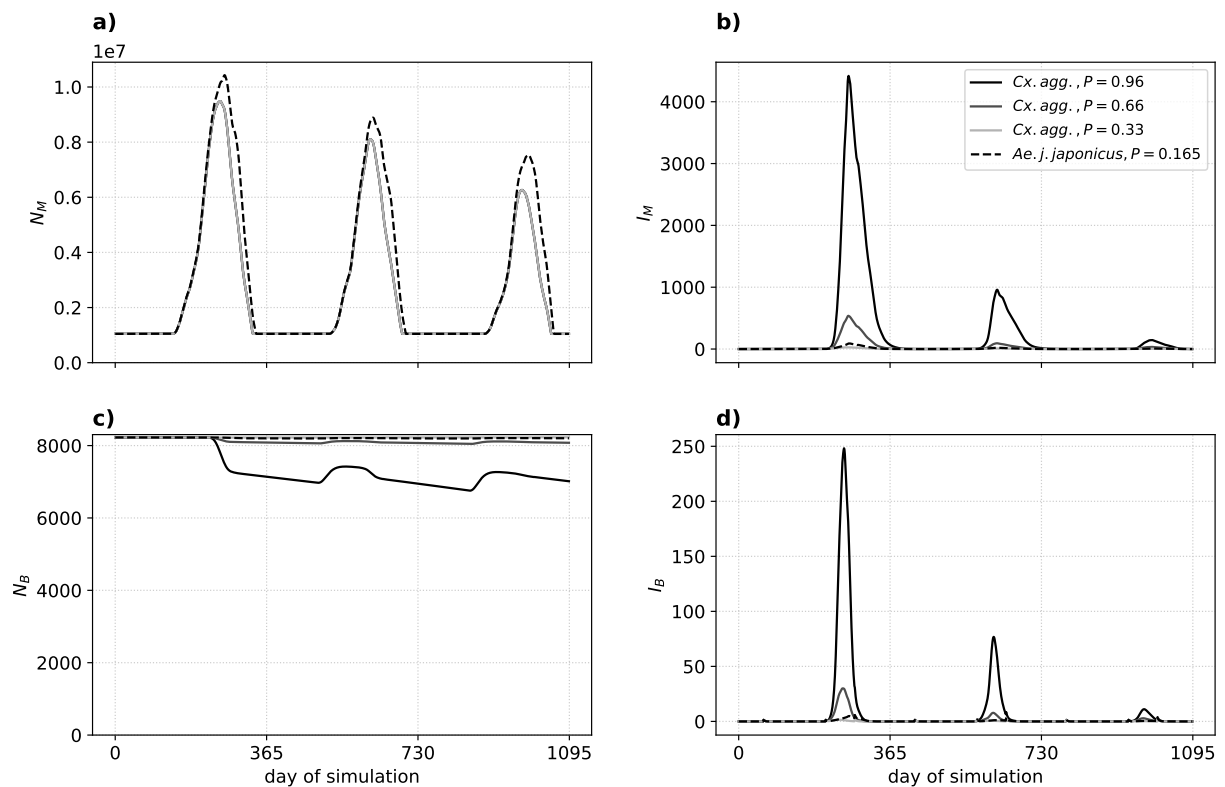
Figure 4.20: Simulation results for the application of the SEIR model to the period 2018-2020 and study region 1. *P* refers to the proportion of blood meals taken on birds.
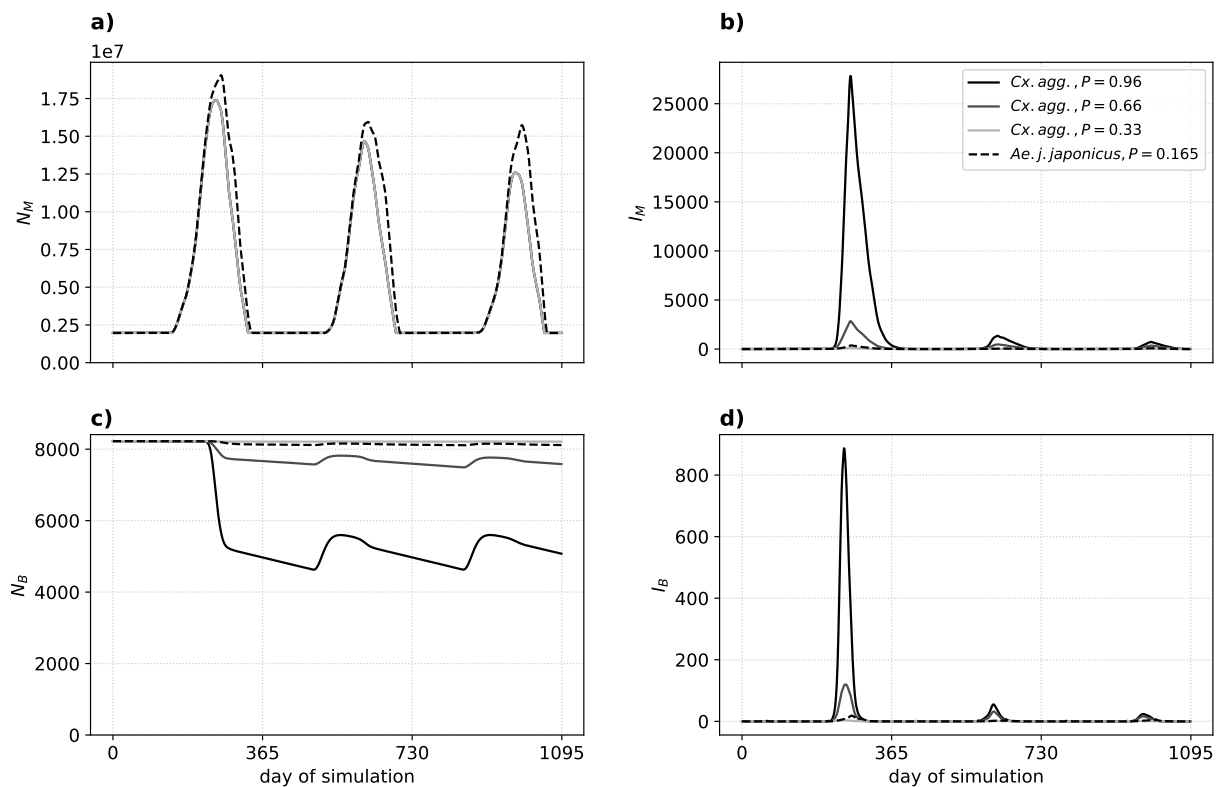
Figure 4.21: Simulation results for the application of the SEIR model to the period 2018–2020 and study region 2. *P* refers to the proportion of blood meals taken on birds.
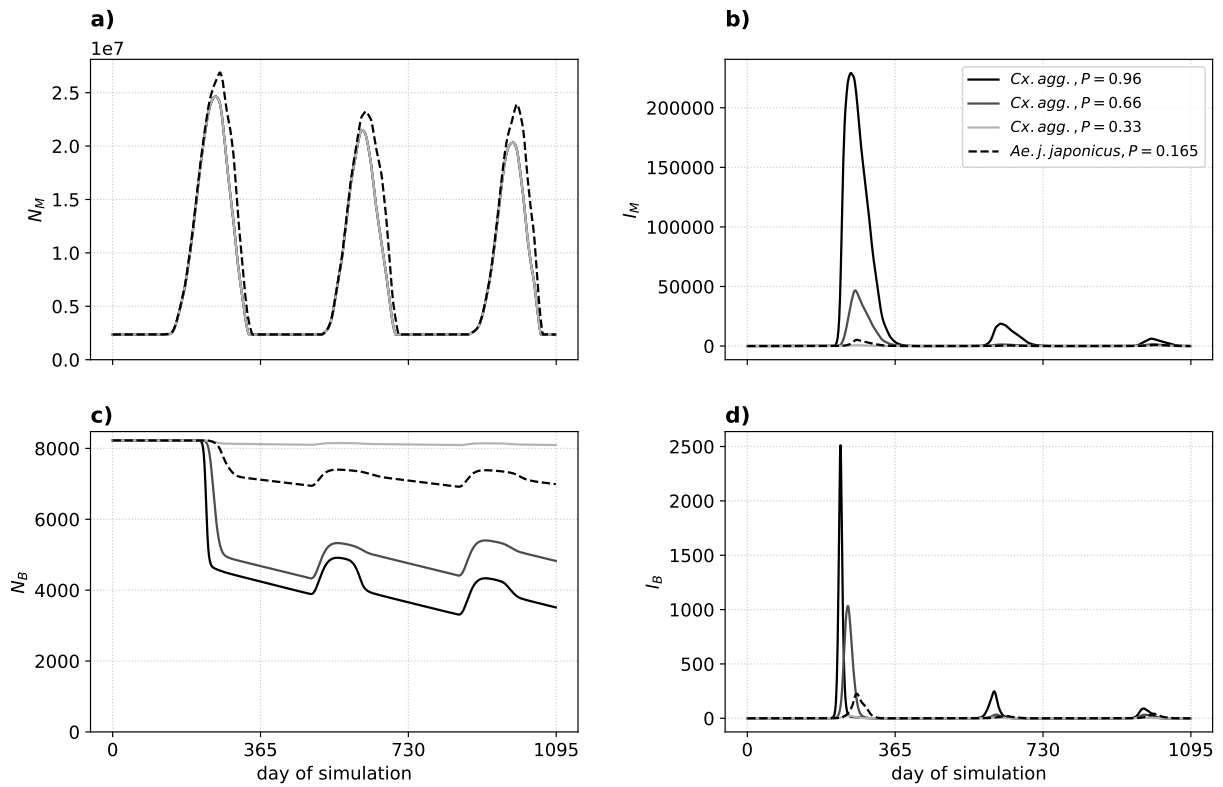
Figure 4.22: Simulation results for the application of the SEIR model to the period 2018-2020 and study region 3. *P* refers to the proportion of blood meals taken on birds.

influence the vectorial capacityof both, *Ae. j. japonicus* and the most important vectors from the species of the genus *Culex*, *Cx. pipiens biotype pipiens*.

The latter aspect includes that data on the virus incubation period among vector mosquitoes in relation to temperature is extremely limited and that the function used here is based solely on an experiment with *Cx. tarsalis*. In addition, the results of laboratory experiments on *Ae. j. japonicus* to investigate the dissemination rate and transmission efficiency for WNV are not consistent. While Huber et al. (2014) [43] found resistance to infection in individuals from south-west Germany and could not identify co-infections with other flavi-viruses or the *Wolbachia* bacterium of the mosquitoes which could bias the results, Veronesi et al. (2018) [40] were able to demonstrate dissemination rates of 15.6 % for strain NY99 and 18.3 for FIN in Swiss individuals. The results of blood meal analyses of the vector mosquitoes do not give a consistent impression either, as they seem to depend on host availability [90]. However, our scenario analyses for the *Culex agg.* show a strong correlation between the degree of ornithophilia and overall vectorial capacity.

Furthermore (v), the carrying capacity of *Ae. j. japonicus* larvae in the study regions is only estimated based on a habitat map derived from a nested modelling approach [70] and on a study indicating the larval abundance in an ideal habitat. The habitat map, however, was not validated with abundance data but with occurrence data mainly derived from the citizen science project "Mückenatlas" [76]. A check of the correlation between abundance data from a systematic monitoring and of the different habitat qualities, as performed for example in the studies of Mushinzimana et al. (2006) [110] and Chaves et al. (2017) [111], is therefore an important project for the future.

It was also assumed that the carrying capacity of larvae of the *Culex agg.* in the respective study regions is identical to that of *Ae. j. japonicus*. We made this setting in order to consider the question of better vectorial capacityapart from vector abundance. Thus, since the regional abundance of mosquitoes from the *Culex agg.* is unknown for the study regions, the intensity of potential epizootics for the *Culex* scenario remains unknown as well.

There are additional factors that can play a role in the development of a WNV epizootic that we have not considered to focus on the essential factors and to not excessively modify the evaluated SEIR baseline model. First, the development of the mosquito population in the model is dependent on the carrying capacity of the larvae, temperature-dependent birth and death rates, and the proportion of non-diapausing mosquitoes as a function of the season. In fact, winter frosts [94] and precipitation [33] also affect population development.

Moreover, there is evidence that some species of mammals and amphibians, e.g. squirrels, rabbits and frogs, are also susceptible to WNV infection and develop high levels of viraemia. This way of transmission, however, is supposed to play only a minor role [1].

The model neglects vertical transmission paths for mosquitoes and horizontal paths for host birds. However, there are indications for vertical (trans-ovarian) transmissions in different species of the *Culex* genus. The transmission efficiency can vary between 10 to 40 % [112]. It is also assumed that vertically infected mosquitoes facilitate the overwintering of the virus in many regions [11, 32, 113, 114]. Birds can become infected horizontally through consumption of infected birds of prey, feather-picking and oral contact with the faeces of other birds. Horizontal transmission in birds could play a major role. It is suspected to be the reason why birds of prey and feather-pickers, such as crows and hawks, become infected in nature frequently [23, 115]. Scenario analyses to investigate the effects of different trans-ovarian transmission rates in mosquitoes and horizontal transmission rates in birds remain an important future task for disease modellers, but adjustments can easily be made in the SEIR model.

The influence of infections of the birds and mosquitoes with other pathogens is also neglected in our model. However, the susceptibility of host birds may be affected by previous infections with viruses from the group of Japanese Encephalitis viruses, which includes the Usutu virus occurring in Germany [23]. The vector competence of the mosquitoes as well as population size and sex ratio can also be influenced by cross-infection with the bacterium *Wolbachia pipientis* [32, 116].

Scenario analyses   It is scientific consensus that many factors determine the vectorial capacity of a mosquito. In addition to the transmission probability, ornitophilic or mammolophilic feeding behaviour is considered a main criterion for the vectorial capacity. Following this theory, vector species are often categorised as "bridge vector" or "maintaining and amplifying vector" [1, 87]. According to the study on host-feeding patterns of mosquito species in Germany [87], however, none of the considered vector species is completely ornitophilic. Instead, it is necessary to speak of a degree of ornithophilia for each species. This degree of ornithophilia can vary greatly between regions, making a general classification of species into these categories very superficial. Also Kilpatrick et al. (2005) [117] argue that the concept is paradigmatic. They showed that even with an extremely high degree of ornithophilia, *Cx. pipiens* can be not only the amplifying vector but also the most significant bridge vector for virus transmission to humans. Our scenario analyses help to assess the influence of the degree of ornithophilia compared to the influence of species-specific transmission probabilities and seasonal abundance.

We were able to show that the proportion of blood meals taken on birds plays an exceptionally important role in defining the vectorial capacity, but that this factor must reach a certain threshold to compensate for differences in transmission efficiency. With a degree of ornithophilia of 33 % for

the *Culex agg.* which is twice that of *Ae. j. japonicus*, this threshold had not yet been reached. This is interesting, as this value reflects the result of the average value of a multi-year, Germany-wide study in which various trapping methods were applied [87]. Thus, in Germany *Ae. j. japonicus* could have a higher average vectorial capacity than the *Culex agg.* when local abundance is equal. However, there seem to be many regions inside and outside Europe, where the species of the *Culex agg.* have an extremely strong preference for birds, and in these regions they are of much greater importance for the outbreak and maintenance of epizootics.

The model applications for the different study regions show that the abundance of vectors also has an extremely strong effect on the development and extent of an epizootic (Fig. 4.20, 4.21, 4.22). Scenario analyses with different abundances of host birds showed even greater effects. The ratio of mosquitoes to birds in the model is a sensible parameter, since a susceptible mosquito must first become infected on a bird and then transmit it to another bird during the blood meal to activate an epizootic. If there are too many birds in relation to the vector mosquito population, the mosquitoes will seek out non-infected birds too often at the start of the WNV season and thus a trans-sylvatic transmission cycle will not start.

In addition to the mosquito abundances, the temperatures in the study regions also differ. The average daily temperatures increase from north to south (Fig. 4.6). On the one hand, this has an influence on the life-trait parameters and the biting rate of the mosquitoes (Fig. 4.8). On the other hand, the temperature shortens the extrinsic incubation period exponentially (Fig. 4.10) so that even small temperature differences in midsummer significantly influence the intensity of the epizootic. That the intensity of the epizootic also differs between years, however, is mainly due to the fact that some birds acquire immunity the first year of the applications, weakening the course of the infection.

4.6.2 Spatial model component

The flight simulator can predict the locations of mosquito hotspots within a region as well as dispersion barriers and stepping stones for potentially infected individuals. The flight simulations can also be used to assess how efficiently the virus can spread from a region to the respective cardinal points through active flights of the mosquitoes. This is useful after a sudden local introduction of the virus as it was the case in 2018 in some places in Germany. Our model is freely available under the Creative Commons Attribution - Non Commercial 4.0 (https://github.com/akerkow/bush-mosquito-flight-simulator) and is based on free software, exclusively. Therefore, it can be adapted to new research results and applied to other regions.

As mentioned in Chapter 4.6.1, the model needs to be extended for other vector mosquito species. We have implemented the flight simulator for the Japanese bush mosquito *Ae. j. japonicus*, since there are yet no comparable high-resolution habitat maps for Germany available for other vector species like *Cx. pipiens*, further species of the *Culex agg.*, or *Ae. vexans*.

The validation of the flight simulator for *Ae. j. japonicus* is difficult. This is often the case for ABM models, as they follow a bottom-up logic and require many detailed and individually validated input parameters for the target species with respect to behaviour and certain characteristics and the respective heterogeneities. These parameters and behavioural rules are integrated into the model to generate an overall picture. However, data on all required parameters and behaviours are often extremely difficult to find in adequate quality. For instance, since no published study on catch-recapture experiments with *Ae. j. japonicus* exists, we have fitted flight distance data for *Aedes albopictus* assuming that this species has a similar flight behaviour.

Although numerous studies provide information on the propagation speed of the invasive Japanese bush mosquito [48, 75, 103, 105, 118], it is not possible to determine the extent of the influence of passive propagation by traffic in any of the observations. Studies suggest that *Ae. j. japonicus* and other invasive species of the genus *Aedes* spread over longer distances by means of transport [105, 119]. However, in our model we have concentrated on the active spread of the mosquito.

Another setting in the flight simulator which is difficult to prove with biological data is the flight motivation of mosquitoes. In our flight simulations, the flight motivation depends exclusively on the habitat qualities at the current location. In fact, mosquitoes can also fly in a targeted manner. Furthermore, the mosquitoes in the flight simulator have an energy level on a defined scale which is filled up during stays in good habitats and reduced by stays in unfavourable habitats, by a general daily consumption and by the flight distances covered. This energy scale and its effect on the mosquitoes are difficult to prove on a biological level and are rather a tool to control the flights of the mosquitoes and create dispersion barriers and stepping stones.

The flight simulator is implemented as an agent-based model. ABMs are computationally intensive and therefore not well suited to deal with large numbers of individuals, such as millions of mosquitoes in our case. However, by bundling several mosquitoes into super-mosquitoes, we were able to bring the run times into an acceptable range of only several minutes with a super-mosquito factor of 1,000. The other side of agent models is that they can be extended in many ways. Thus, additional host bird and vector mosquito species, each with different virus transmission characteristics, could be added in the future, resulting in a multi-agent model.

4.7   Conclusions

We have adapted a WNV compartment (Susceptible-Exposed-Infectious-Removed) model for both a potential host bird and vector mosquito species (*Pica pica* and *Aedes japonicus japonicus*) occurring in Germany and coupled it with a flight simulator for the vector mosquitoes. The flight simulator is an agent-based model built on a habitat map for the mosquito species being invasive in Germany, data on local temperature and wind conditions, and studies on the flight behaviour of this and other mosquito species.

The spatial extension of the SEIR model is the first step in developing a tool that can estimate for a region (i) whether an epizootic can emerge after virus introduction, (ii) how large the vector mosquito population is, and (iii) where vector mosquito hot spots, dispersal corridors, stepping stones and barriers are located. In addition, (iv) the model can calculate the probability of infectious mosquitoes leaving the region in the respective cardinal directions. The model extension is not only a visualisation tool, but also intervenes in the differential equations of the SEIR model. This applies in particular to regions with few suitable habitats, where the potential size of the mosquito population is reduced on a daily basis as a result of long flight distances.

Since many bird and mosquito species are hosts and vectors of West Nile virus, the current version of the model cannot yet reliably determine the extent of an epizootic. In a next step, it must be expanded to include other important bird and mosquito species with their respective life-trait parameters, virus incubation times, transmission rates, and so forth.

The spatially extended WNV model is a tool aimed at modellers involved in planning control measures for WNV vector mosquitoes. It is particularly useful for applications in regions where the virus has been recently introduced. For model applications, we recommend selecting the size of the study area to cover the territories of the host birds surrounding the reported WNV cases. Local ornithologists may be consulted to assist with this.

We demonstrated the model linked with the flight simulator by applying it to three study regions in Germany. The regions differ in terms of weather conditions, habitat structure and average habitat quality for *Ae. j. japonicus*. Using model applications without spatial coupling, we further analysed whether *Ae. j. japonicus* has a higher vectorial capacity than a *Culex* species aggregate of the same population size. We were able to show that no general answer can be given to this question, as it depends on the degree of ornithophilia which can vary greatly between different localities.

Abbreviations

***ABM* :** Agent-based model
***Ae. j. japonicus; Aedes j. japonicus* :** *Aedes japonicus japonicus*
***Culex agg.* :** Aggregate of species from the genus *Culex* (see Chapter 4.3.5)
***Cx.* :** *Culex*
***P. pica* :** *Pica pica*
***WNV* :** West Nile virus

Acknowledgements

Supplementary materials

S1, S2, S3:
Videos showing the 2D-movements of female super-mosquitoes (n=10) in all stages of infection in study region 1 (S1), 2 (S2) and 3 (S3), respectfully. The videos are derived from model applications to the year 2018.
S4, S5, S6:
Videos showing the 2D-movements of infectious super-mosquitoes (n=10) in study region 1 (S4), 2 (S5) and 3 (S6), respectfully. The videos are derived from model applications to the year 2018.

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Chapter 5

General Discussion

The focus of this work was the development of a new methodology for habitat modelling of invasive mosquito species, and its application to the invasive Asian bush mosquito *Aedes japonicus japonicus* in the region of Germany. Furthermore, this work includes a simulation of the active flight movements of *Ae. j. japonicus* linked with an infection model of the West Nile virus. All sub-models presented can later be adapted to other native or invasive mosquito species that are also relevant, for example, from an infection biology perspective.

5.1   Steps in the habitat modelling process

The presented procedure for building the habitat model is carried out in several steps:

5.1.1   Step 1: Climate model

Mesoclimate data of $1 \, \text{km} \times 1 \, \text{km}$ resolution were used to implement a climate model. The development of a climate model for a recently invaded species is particularly challenging, as the amount of data at this early stage of invasion is limited and the new distribution area does not yet reflect the range of all environmental conditions acceptable to the species. Therefore, a data-driven species distribution model will likely calculate the potential colonisation area to be too small.

In addition to analysing the climate in regions where a species occurs (in this case the invaded areas), it is equally beneficial from a modelling perspective to analyse where the species does not occur. In other words, absence data can greatly improve species distribution models [1]. For only recently established species, however, absence data cannot be used to improve the model because

it is unclear whether the species was not mapped at a location because (i) it cannot occur there due to its environmental requirements, (ii) it has been overlooked, or (iii) it could actually occur there but has not yet spread to that location.

Since niche modelling for an invasive species based on the exclusive use of occurrence data (so called presence data) underrepresents regions suitable for dispersal, and since absence data cannot be reliably generated for invasive species, the challenge faced in climate modelling was to find a suitable approach for training the available presence data.

The climate modelling procedure was optimised in several steps. First, the basic methodology for identifying the realised climatic niche of *Ae. j. japonicus* was developed. The method involves using presence data of this species, as well as presence data of three native species (instead of absence data of the target species), to analyse the habitat of the target species with the help of machine learning. Furthermore, a procedure for selecting the climate variables to be integrated into the model was developed as part of the optimisation process. Using the genetic algorithm DEAP [2, 3] and cluster computing, those 8 out of 37 pre-selected variables were filtered out that achieved the best results in the model trainings and thus could best describe the partly different distribution areas of the species [4]. Finally, the impact of different classification algorithms on the climate model was investigated [5]. Four different algorithms (Support Vector Machine, Random Forest, Logistic Regression and Decision Tree) were used and also combined with each other to create so-called "ensemble models" [3].

In order to decide which algorithms perform better compared to others, the model results were evaluated using the parameters "exactness" and "selectivity". Based on these values, the algorithms were ranked partially by means of a Hasse diagram [5]. It resulted that the Support Vector Machine performed best in the validation of all the individually-applied algorithms, but some ensemble models performed even better. The Support Vector Machine [6, 7] is a black-box modelling approach, which means that the decisions underlying the classification remain unknown.

The fact that modeling the climatic niche (Step 1) consists of many time-consuming substeps could be an obstacle to establishing it as a standard method to be applied to invasive species. In particular, the DEAP learning procedure for selecting the climate parameters is time-consuming and requires enormous computational power [4]. However, it has been shown that the feature selection process is a key factor in generating well-performing models, and that biological expert knowledge about mosquitoes in general and the species to be modelled cannot compete with computer-based optimisation methods [4, 8].

In addition, it is not easy even for a mosquito expert to assess whether, for example, the average temperature in summer (average of several months) or in August has a greater influence on the presence or absence of a species. Climate data exist in huge numbers and with many different temporal resolutions, and many parameters correlate with each other. Furthermore, it is almost impossible to know which realised ecological niche a species occupies in nature, because predation and competition with other mosquito species may also play a role. By replacing absence data of the target species with presence data of other mosquito species, pondering about the appropriate climatic variables becomes even more complicated. An alternative for including occurrence data of mosquito species other than the target species in the model would be to integrate pseudo-absence data (so-called background data) [9].

Training a model with occurrence data of several other mosquito species instead of absence data is, as far as I know, not yet an established procedure. However, there are already a few approaches in which data on the occurrence of other species were integrated into the model as predictor variables [10, 11]. In principle, the selection of mosquito species best suited for analysing the ecological niche of the target species could also be done by applying optimisation procedures, as there are around 50 mosquito species in Germany alone [12]. During the development process of the model presented in this work, only a few combinations of mosquito species were tested, also with different numbers. In doing so, it turned out that the integration of too many as well as too few species negatively influenced the model training as evaluated by means of confusion matrices [13].

When creating a distribution model for an invasive mosquito species, the principle that a species must be in equilibrium with its environment in order to be modelled is always being disregarded [11, 14, 15]. If, instead of absence data, data from several other mosquito species are included, and if at least one of the species is located exclusively in remote locations far away from the invasion area of the target species, then the possible dispersal potential of the invasive species might be particularly underestimated. This effect may be present in the presented climate model application due to the inclusion of the species *Aedes daciae* in the model.

5.1.2   Step 2: Fuzzy-based habitat model

The climate model was combined with landscape and wind speed data in a nested approach and by using the fuzzy modelling technique [16]. Many different large-scale land use parameters, such as field, pasture and forest areas, but also small-scale landscape details, such as the occurrence of cemeteries, zoos and allotment gardens, are taken into account. The landscape data can effectively

describe the occurrence of mosquitoes on a small scale, as the landscape has an influence on the micro-climate and is an indicator for the presence of possible breeding habitats.

With the help of fuzzy rules, many considerations about the ecology of *Ae. j. japonicus* could be taken into account. For example, it was possible to define that unfavourable climatic conditions can be partially compensated by a very suitable form of land use. If, for instance, a place has very little rainfall, this is unfavourable for the occurrence of the species, as rain is important for filling the typical small artificial breeding containers and tree holes. However, near settlements or in cemeteries, it can be assumed that precipitation is irrelevant, since flower vases and plant tubs are filled with water by humans, and wells or irrigation systems are available. The output of the nested model with its $100\,\mathrm{m} \times 100\,\mathrm{m}$ resolution is very detailed and performs significantly better in the validation than the models based solely on mesoclimate data.

Applying fuzzy logic was a good way to combine expert knowledge and machine learning, as well as data with different resolutions. The integration of expert knowledge was essential in our approach to compensate for the weaknesses of machine learning methods resulting from training with biased data from passive mosquito monitoring by citizens as part of the project "Mückenatlas".

The disadvantage of the fuzzy method is that it is more complex to implement than a data-driven machine-learning approach. Experts must be consulted or a literature or laboratory study must be conducted. In addition, many rules have to be established regarding the effects of combining certain environmental conditions. A concern here is that expert knowledge tends to be biased by subjective perception, and laboratory experiments can only be used to determine physiological tolerance limits of animals, which describe the fundamental niche of a species and not the realised ecological niche. In addition, laboratory experiments are often conducted under constant conditions that do not occur in nature.

An interesting project for the future in this context would be to integrate the climate, wind and landscape data into one machine learning procedure, making the fuzzy modelling step unnecessary. To achieve this without loss of quality due to training biases, especially with respect to the land use variables, studies on the exact biases of the training data [17, 18] can be consulted and, based on the results of these studies, the mosquito occurrence data can be selected for training in a way that compensates for these biases. However, it can be assumed that the model quality is not significantly improved by selecting the species occurrence data, because species distribution models are not only very sensitive to data biases, but also to the sample size of the data [8].

When selecting the relevant set of landscape parameters for the landscape model (as input to the fuzzy model), I conducted a literature review and investigated possible environmental

variables associated with the species using geo-analysis techniques. In doing so, I had to disprove the hypothesis that the occurrence of *Ae. j. japonicus* depends on altitude [19–22] for the area of Germany, but when looking at the mosquito data in combination with relief data, I noticed that *Ae. j. japonicus* is much more frequent in valleys than on hills. I assumed that there was at least one other parameter that could describe this pattern and significantly contribute to the ecological niche of the species, and my attention was drawn to wind data.

Mean wind speed data could explain the described pattern of distribution stunningly well by visual inspection, and its inclusion in the fuzzy model significantly improved model selectivity and exactness [5]. This was surprising because, although it is known that wind strongly influences the flight behaviour of numerous mosquito species [23], there was, to the best of my knowledge, not a single species distribution model for mosquitoes at the time of modelling that took wind data into account as a predictor variable. Also among the 37 environmental variables from which the training data set was selected with the help of the DEAP learning algorithm, there were no wind data but only temperature and precipitation data, data on the number of frost days and drought indices [4].

5.2   Results of the species distribution models

The calibrated version of the climate model where the training is based on long-term climate data from the period 1981–2010 and an updated set of field collection data from 2012–2017 (compare Chapter 3.3.2) was validated by reserving 10 % of the mosquito collection data for 40-fold cross validation. The model reached an accuracy of 84.13 % in the training with a standard deviation of 1.22 % and the hit rate (median prediction value at the intersections of the climate suitability map with the occurrence points of *Ae. j. japonicus*) was 0.78 %. This result indicates a good prediction power of the climate model. By combining the results of this climate map with wind data and the data from the landscape model, the hit rate could be increased significantly to 0.86 % without any decline in model selectivity.

Unfortunately, the model results presented here cannot be directly compared with those of other models for *Ae. j. japonicus* in Germany that are based on machine learning [15, 24]. This is because they were validated using a different method, namely the AUC (area under the curve [25]), which requires absence data or so-called background data [25]). In addition, the models are hardly comparable with each other in terms of quality because they use training data from different sources and years, and also map the results to different climate periods. In addition, the set of climate variables for the training of the climate models was different. When comparing

the prediction maps on the basis of the climate data, however, the model results for the declared current climate period differ only slightly from each other, despite all the differences.

Considerable differences in the prediction of climatically suitable regions, however, can be seen when comparing the results of our climate model with the model results for the current climate period (1993–2013) derived from experimental life history data obtained from *Ae. j. japonicus* specimens from southern Germany [26]. The life-history model predicts a wider range of distribution than the calibrated climate model presented in this work. This could indicate that the climate model presented here underestimates the climatic niche due to a data set of the invasive species that is still too small. Another possible reason for this finding might be that a life-history data-based model represents the fundamental niche of the species, which does not take into account the effect of other environmental factors, e.g. the presence of predators or competitors.

5.3   Mosquito flight simulator as a spatial extension to a West Nile virus compartment model

In addition to analysing habitats and creating distribution maps of medically relevant mosquito species, infection models are also of great importance for society and the protection of animals at risk from emerging arboviruses. As detailed habitat maps for *Ae. j. japonicus* are available from the preceding works, I have been able to build on this and add a spatial component to an infection model. The implementation of a West Nile virus transmission model was of particular interest, as *Ae. j. japonicus* has shown high dissemination and transmission rates as well as a short extrinsic incubation period (EIP) for the virus in some laboratory experiments [27–30]. The first two parameters describe the vector competence, "the inherent capacity of an invertebrate host to become infected and ultimately transmit a given Pathogen" [31], and all three are important factors for the vectorial capacity. The vectorial capacity describes the function of a species as a vector as a whole and, in addition to the parameters of vector competence, includes i.a. the EIP, abundance and survival of mosquito females, as well as the host preference and blood feeding patterns [32].

West Nile virus (WNV) is transmitted by several mosquito species to birds, humans or other mammals, but only birds act as hosts and thus maintain the transmission cycle of the virus [33]. Using an existing WNV infection model implemented for an agglomerate of species of the "Culex" genus [34] that I adapted to the Asian bush mosquito and a host bird species living in Germany, I addressed the question of the proportion of blood meals that *Ae. j. japonicus* needed to take

on birds in order to (i) trigger epizootics under appropriate weather conditions and (ii) have a higher vectorial capacity than the *Culex* species aggregate. So far, *Ae. j. japonicus* has not been considered an amplifying and maintaining vector for the virus despite laboratory experiments suggesting a high vector competence (at least of some populations) of the species [35–40]. This assessment is based on mammolophilic feeding behaviour, but in fact the exact vectorial capacity of *Ae. j. japonicus* for WNV has not yet been calculated or modelled for any region. However, the relevant studies suggest a possible function of *Ae. j. japonicus* as a bridge vector for the transmission of the virus to mammals and humans, which are dead-end hosts for the virus.

I prepared scenario analyses by applying the compartment (SEIR) model to both, *Ae. j. japonicus* as well as the species of the *Culex* aggregate as defined in the models of Rubel et al. (2008) and Laperriere et al. (2011) [34, 41]. The scenario analyses help to assess the influence of the degree of ornithophilia compared to the influence of species-specific transmission probabilities and seasonal abundance. Three different values for the degree of ornithophilia were used for the *Culex* agg. because it can vary greatly by region [42, 43] and also differs depending on the exact species (and biotype) composition of the *Culex* complex [44].

The applications revealed that the proportion of blood meals taken on birds must overpass a threshold of 33 % for the *Culex* agg. in order to reach the same level of vectorial capacity compared to that of *Ae. j. japonicus* and therefore compensate for differences in transmission efficiency. This is a very interesting result considering the fact that Börstler et al. (2016) [42] found the transmission rate of species from the *Culex* genus to be only 33 % on average. They conducted a multi-year, Germany-wide study in which various trapping methods were applied. Therefore, in Germany *Ae. j. japonicus* could have a higher average vectorial capacity than the *Culex agg.* when local abundance is equal. In order to make more precise statements on the comparison of vector capacities, it would be beneficial if studies such as those mentioned above [42] could include the local abundances of all mosquito species caught, so that the actual vectorial capacities can be determined.

In order to enable the scenario analyses for the mosquito species with regard to the degree of ornithophilia and to be able to apply the model with estimated realistic abundance data for the mosquitoes, the factors "proportions of blood meals taken on birds" and "proportion of bird bites taken on a host bird" had to be integrated into the equations in the adopted compartment model. The importance of considering these factors is supported by a study by Simpson et al. (2012) [45], which created an SIR model for regions affected by WNV occurrence in Connecticut (USA) with *Culex pipiens* and integrated measurements of blood meal sources.

The West Nile virus compartment model has been extended by a spatial component, an agent-based flight simulator for the Asian bush mosquito which simulates spatial (2D) flight movements within study regions and thus identifies where the mosquitoes are likely to accumulate over time and where most of the infectious mosquitoes are likely to be. In the future, the study region could be, for example, a region where WNV infections have recently occurred. In the applications presented herein, the study regions are located in well-known occurrence areas of *Ae. j. japonicus* in Germany and have an extent of $625\,\text{km}^2$. The spatially extended WNV model can also calculate and display the position of the infected mosquito agents. However, since the mosquitoes in the spatial model move with a random component, and since the model is based on the assumption of unrestricted movements of the birds within the region, the position of the infected mosquitoes is different each time the model is run.

The application of the coupled model to three different regions has, in addition to generating different temperature scenarios, enabled the formula for the energy balance in the flight simulator to be set approximately. The energy level in the model can cause mosquitoes to die after a longer stay in unfavourable habitats. This affects the abundance of mosquitoes in the region, and the abundance in turn affects the infection equations. The balancing of the factors of the energy equation was made possible by approximate descriptions of population developments in the respective regions [46, 47].

Unfortunately, there are no suitable abundance data for *Ae. j. japonicus* for any region in Germany, so the flight simulator could not be validated properly and the corresponding parameter in the WNV compartment model could only be set with uncertainty. Studies on the abundance (density per unit area) of mosquito larvae or imagoes are scarce because they are very time-consuming, especially for larger regions. More common are measurements of relative population sizes, such as the container index (percentage of containers with larvae), the Breteau index (number of active breeding sites per 100 premises) or the ovitrap index (the average proportion of ovitraps with mosquitoes) [48]. Finding a way to generate a map of maximum larval abundance from the habitat map generated in the preliminary work was an important step for implementing and scaling this model.

If appropriately comparable study results, such as those of Ibañez-Justicia et al. (2018) [49], become available for different regions and habitat types in Germany in the future, the method described in this dissertation in Chapter 4, or machine learning methods as described in Gervasi et al. (2019) [50], can be used to generate an abundance map for *Ae. j. japonicus*. Abundance maps offer a significant advantage over habitat maps, especially with regard to the development of transmission models of mosquito-borne diseases such as WNV.

5.4 References

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Appendices

Appendix A

Statement of Academic Integrity

I hereby declare that I have written the dissertation presented here independently without the assistance of any third party, that I have not used any sources or auxiliaries other than those indicated, and that I have marked any quotations, whether verbatim or in spirit, as such. All collaborations with other scientists and their contributions to the published articles as well as the article whose submission is in preparation are truthfully stated in the "List of Publications with Author Contributions".

This thesis has not been submitted in the same or a similar form to any other examination authority and has not yet been published, I have not applied for a doctoral degree at another university in Germany or abroad or at another department at any time in the past, and I am aware of the doctoral regulations at Freie Universität Berlin.

Appendix B

Acknowledgements