UNIVERSITÄT BAYREUTH

Department of Biogeography

Master thesis (MSc Geoecology)

Modeling the Risk of Dengue Fever in Europe Based on the Extrinsic Incubation Period and Climate Change Projections

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Abstract

Globally, dengue fever is one of the most rapidly spreading vector-borne-diseases due to increasing trade as well as travel and changing environmental conditions with advancing climate change. Furthermore, the establishment of the vector *Aedes albopictus* SKUSE continues to progress in Europe. The time interval between virus acquisition and transmission (EIP) by the vector is negatively correlated with temperature. Thus, it was hypothesized that the climatic suitability for dengue transmission will increase in Europe with advancing climate change. For modeling the relationship between temperature and EIP of dengue, a non-linear unimodal function was applied. Subsequently, the EIP and the climatic suitability for *Ae. albopictus* in Europe were modelled based on temperature parameters. Both models were combined to project the climatic suitability for dengue transmission in Europe. All projections referred to the 21st century and were based on two divergent IPCC5 scenarios (RCP4.5 and RCP8.5). The EIP of dengue was detected to shorten generally in Europe in times of climate change. The climatic suitability for *Ae. albopictus* was projected to become higher in Europe. Consequently, the potential for dengue transmission was detected to increase in Europe with advancing climate change. Areas with high climatic suitability were projected to expand and to show a north-ward shift. Moreover, south-western parts of the Iberian Peninsula are expected to show the shortest EIP in Europe, but a low potential for dengue transmission due to a low climatic suitability for *Ae. albopictus*. Hotspots for dengue transmission were projected for the Mediterranean islands and for the coastline along the Mediterranean Sea as well as Black Sea. Thus, especially touristic areas are expected to be hotspots for dengue transmission. Here, the transmission risk for dengue can be facilitated through increasing human population density and increasing travel activity during summer. Thus, monitoring and control measures should be set up to recognize dengue outbreaks and to avoid the spread of dengue across Europe. Dengue is no longer a mere tropical disease. In the near future it can become a threat for Europe with advancing climate change.

Zusammenfassung

Aufgrund von zunehmendem Handel und Reisetätigkeiten sowie sich verändernden Umweltbedingungen im Zuge des Klimawandels zählt Dengue-Fieber zu den vektorübetragenen Krankheiten, die sich weltweit am schnellsten ausbreiten. Darüber hinaus schreitet die Etablierung des Vektors *Aedes albopictus* SKUSE in Europa immer weiter voran. Die Zeitspanne, die zwischen Virus Aufnahme und der Möglichkeit der Weitergabe (extrinsische Inkubationszeit) durch den Vektor liegt, korreliert negativ mit der Temperatur. Daher wurde die Hypothese aufgestellt, dass die klimatischen Bedingungen, die für eine Übertragung von Dengue-Fieber geeignet sind, in Europa im Zuge des Klimawandels zunehmen werden. Die Beziehung zwischen Temperatur und der extrinsischen Inkubationszeit von Dengue-Fieber wurde modelliert, indem eine nicht-lineare unimodale Funktion angepasst wurde. Anschließend wurde die extrinsische Inkubationszeit und die Eignung der klimatischen Bedingungen für *Ae. albopictus* auf Grundlage von Temperaturparametern modelliert. Beide Modelle wurden kombiniert, um die Eignung der klimatischen Bedingungen für Dengue-Fieber in Europa zu modellieren. Alle Projektionen beziehen sich auf das 21. Jahrhundert und basieren auf zwei unterschiedlichen IPCC5 Szenarien (RCP4.5 und RCP8.5). Es konnte festgestellt werden, dass sich die extrinsische Inkubationszeit in Europa im Zuge des Klimawandels verkürzen wird. Die klimatischen Bedingungen in Europa werden zunehmend den Anforderung von *Ae. albopictus* entsprechen. Es konnte weiterhin festgestellt werden, dass sich das Potential für eine Dengue-Übertragung in Europa im Zuge des Klimawandels erhöhen wird. Laut Projektionen werden sich Gebiete mit sehr geeigneten klimatischen Bedingungen ausdehnen und nach Norden verlagern. Weiterhin wird erwartet, dass der südwestliche Teil der iberischen Halbinsel durch die kürzeste extrinsische Inkubationszeit in Europa gekennzeichnet ist. Aufgrund einer geringen klimatischen Eignung für *Ae. albopictus* wird der südwestliche Teil der iberischen Halbinsel jedoch ein geringes Potential für Dengue-Übertragung aufweisen. Brennpunkte einer Dengue-Übertragung werden laut Projektionen die Mittelmeerinseln, sowie die Schwarzmeer- und Mittelmeerküste sein. Daher werden in Zukunft insbesondere touristische Gebiete solche Brennpunkte sein. Zusätzlich kann sich hier die Gefahr einer Dengue-Übertragung durch ansteigende Bevölkerungsdichte und Reiseaktivität im Sommer weiter erhöhen. Daher sollten Überwachungs- und Kontrollmaßnahmen eingeführt werden, um Dengue-Ausbrüche zu erkennen und die Ausbreitung über Europa zu verhindern. Dengue-Fieber ist nicht mehr nur eine Tropenkrankheit, sondern kann mit fortschreitendem Klimawandel in naher Zukunft zu einer Bedrohung für Europa werden.

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List of Acronyms

AIC .... Akaike Information Criterion
CHIK .... Chikungunya
CHIKV .... Chikungunya Virus
DENV .... Dengue Virus
DENV-1 .... Dengue Virus Serotype 1
DENV-2 .... Dengue Virus Serotype 2
DENV-3 .... Dengue Virus Serotype 3
DENV-4 .... Dengue Virus Serotype 4
EIP .... Extrinsic Incubation Period
GCM .... Global Climate Models
RCP .... Representative Concentration Pathways
RCP4.5 .... Representative Concentration Pathway 4.5 (4.5 $W\,m^{-2}$)
RCP8.5 .... Representative Concentration Pathway 8.5 (8.5 $W\,m^{-2}$)
RSE .... Residual Standard Errors

1 Introduction

In times of global change, the importance of vector-borne-diseases is globally increasing. Increasing temperatures and changes in the precipitation patterns are expected due to climate change (IPCC 2013). Depending on the selected Representative Concentration Pathways (RCP) scenarios and on the algorithm of the chosen Global Climate Model (GCM), a global mean surface temperature increase of 0.3 °C to 4.8 °C is projected for the period from 2081 to 2100 relative to the period from 1850 to 1900. Such climatic changes can have an impact on the development rates, oviposition rates as well as on the survival rates of mosquitoes, and on the pathogens' incubation periods (Williams *et al.* 2014). The spread of mosquitoes as vectors for tropical and subtropical diseases is not just enhanced by changing climatic conditions, but also by the increasing global travel and trade (Jelinek 2009; Simmons *et al.* 2012; Semenza *et al.* 2014). Furthermore, more and more people are affected by vector-borne-diseases such as dengue in the tropics (Jelinek 2009). Thus, the spread of vector-borne-diseases via viraemic travelers into countries, where respective viruses are presently non-endemic, is also increasing (Wilder-Smith & Schwartz 2005; Simmons *et al.* 2012; Leder *et al.* 2013). Among emerging infectious diseases (EIDs), vector-borne-diseases account globally for one third of EID events (Jones *et al.* 2008).

1.1 Dengue fever as important vector-borne-disease

Among vector-borne-diseases, dengue fever is one of the most rapidly spreading and most important vector-borne-disease worldwide (Wilder-Smith *et al.* 2014). Dengue virus (DENV) is transmitted to hosts such as humans by *Aedes* species (World Health Organization 2009). According to the World Health Organization (2009), the estimate for annual dengue infections ranges between 50 million and 100 million worldwide. Recent model estimates result even in 390 million infections per year (Bhatt *et al.* 2013). In 2013, 2.35 million dengue cases were noted just in the Americas (World Health Organization 2014). Of these cases, more than 37 000 were severe dengue cases. An outbreak of dengue can lead to large epidemics, such as the epidemic in the Southern Cone (Argentina, Brazil, Chile, Paraguay, and Uruguay) reported in the first three months in 2015 (Centers for Disease Control and Prevention 2015; Pan American Health Organization 2015; Zanzara Tigre OnLine 2015). So, in this region, more than 450 000 dengue cases (including 235 severe dengue cases) and 132 lethal cases have been reported to the Pan American Health Organization in 2015 (Pan American Health Organization 2015). These outbreaks indicate a serious threat of dengue fever to public health.

DENV is an arthropod-borne RNA virus of the genus *Flavivirus* (Schmidt-Chanasit *et al.* 2010; Simmons *et al.* 2012) that is presently endemic mainly in the tropics and sub-tropics such as Latin America, the Caribbean, Africa, South and Southeast Asia (La Ruche *et al.* 2010; World Health Organization 2014; Wilder-Smith *et al.* 2014). Before 1970, the virus was endemic in nine countries; presently it is endemic in more than 100 countries (World Health Organization 2014).

There are four serotypes of DENV (DENV-1 to DENV-4) with different abilities of geographical dispersion and with specific pathogenity (Halstead 2008). An infection with one of the four serotypes can lead to a serotype-specific lifelong immunity (Simmons *et al.* 2012). Uncomplicated forms of DENV show symptoms such as fever, headache, nausea, vomiting, a rash and muscle as well as joint pains (World Health Organization 2014). There are also severe forms (called severe dengue) such as dengue haemorrhagic fever and dengue shock syndrome with higher mortality rates in average of about 2.5 % (Patz *et al.* 2005; World Health Organization 2014). Severe dengue can be initiated by a secondary infection via other serotypes than the serotype responsible for the first infection (World Health Organization 2014). Symptoms can be plasma and vascular leaking, fluid accumulation, persistent vomiting, severe bleeding such as mucosal and gums bleeding as well as blood in the vomit, respiratory distress such as rapid breathing, increasing abdominal pain, restlessness and damages to organs (World Health Organization 2014). Here, an appropriate medical care and hospitality is required, which can reduce the mortality rate from 20 % to less than 1 % (Patz *et al.* 2005; World Health Organization 2014). In general, there is neither a specific treatment in case of dengue infection nor is a vaccine against dengue yet developed (World Health Organization 2014).

1.2 *Aedes albopictus* as vector for dengue in the temperate climate

Aedes albopictus SKUSE (syn. *Stegomyia albopicta* SKUSE) is listed among the 100 of the World's Worst Invasive Alien Species (Crans 2008). Schaffner *et al.* (2013) and Petrić *et al.* (2014) define "invasive mosquito species [...] by their ability to colonies new territories". Once introduced and established in their new territory, they can change the environment as well as damage human economy and health (Schaffner *et al.* 2013). Invasive mosquito species like *Ae. albopictus* can overcome long distances by passive dispersion such as dispersion by globally traded goods (Medlock *et al.* 2012; Petrić *et al.* 2014). After the introduction of an invasive mosquito species by trade and travel, a successful reproduction and further spreading depends on population parameters such as longevity and breeding behavior as well as on environmental parameters such as climate and land use (Petrić *et al.* 2014).

1.2.1 Ecology of *Aedes albopictus*

The common habitat of *Ae. albopictus* (Fig. 1) is in suburban and rural areas, as some vegetation is required (Hawley 1988; Mitchell 1995). Tree holes or artificial containers such as catch basins, tanks and tires are used as breeding sites (Hawley 1988; Mitchell 1995). Hence, the mosquito is able to use diverse microhabitats for oviposition (Hawley 1988). According to Scholte & Schaffner (2007), the eggs of *Ae. albopictus* are characterized by a high desiccation tolerance. The ability for oviposition in artificial containers in combination with the desiccation tolerance of the eggs leads to the possibility for passive dispersal over long distances (Scholte & Schaffner 2007; Scholte *et al.* 2008). Further, *Ae. albopictus* is feeding mainly on mammals, but can also feed on birds.

It is mainly active during daytime (Hawley 1988; Mitchell 1995). As an ectothermal organism, *Ae. albopictus* is reliant on the climatic conditions in the habitat due to a lacking regulation of the body temperature (Fischer *et al.* 2011). During summer, the optimal temperature for the development rate of *Ae. albopictus* is between 25 °C and 30 °C and the mean temperatures in January should not be below 0 °C for overwintering (Medlock *et al.* 2006; Straetemans 2008). Further, low precipitation, especially during summer, can be a limitation for the breeding success (Straetemans 2008). Hence, it is unlikely that *Ae. albopictus* can colonize areas with less than 300 mm precipitation per year, although *Ae. albopictus* can breed in artificial containers filled with some water (Mitchell 1995). In contrast to Mitchell (1995), Straetemans (2008) stated that an annual precipitation of at least 500 mm is required. The season for *Ae. albopictus* could last from May to November along the northern Mediterranean coast (Schmidt-Chanasit *et al.* 2010).

Fig. 1: *Ae. albopictus is the important vector for dengue in Europe. A female Aedes albopictus biting a human host. Picture by: James Gathany, Centers for Disease Control and Prevention (2002).*

Since *Ae. albopictus* can overwinter in egg diapause, potential habitats are not only restricted to the tropics, but can also be found in temperate regions (Mitchell 1995; Scholte & Schaffner 2007; Thomas *et al.* 2012). The diapause of eggs is induced by low temperatures and photoperiod (Mitchell 1995; Fischer *et al.* 2011). Mogi (2011) found that cold hardiness was increased in more northerly distributed species of *Aedes* compared to more southerly distributed species. Intraspecific differences in cold hardiness could be found for *Ae. albopictus*, which is distributed over a large latitudinal range. According to the study by Thomas *et al.* (2012), populations that had been adapted to cold climate maintained a higher hatching success after frost exposure than populations that had not been adapted to cold climate. In general, eggs of European populations tolerated a higher degree of frost (temperatures of -10 °C) and showed higher hatching success than eggs of tropical populations (tolerating temperatures of -2 °C) after a long term exposure to frost (Thomas *et al.* 2012). Thus, European populations are better adapted to temperate climate than populations from tropical Asia.

Summing up, *Ae. albopictus* is strongly influenced by ecological and human parameters such as availability of artificial containers filled with some water, photoperiod, temperature, and humidity. Here, European *Ae. albopictus* populations are better adapted to the cold period in Europe than tropical *Ae. albopictus* populations. Further, the ability of *Ae. albopictus* to overcome long distances, to breed in various microhabitats and to be an opportunistic feeder with a broad host range leads to a high risk of invasion in new areas.

1.2.2 Vectorial competence of *Aedes aegypti* and *Aedes albopictus*

In general, *Aedes aegypti* L. (syn. *Stegomyia aegypti* L.) is the primary vector for dengue and more efficient in transmission compared to *Ae. albopictus*, which is the secondary vector (Lambrechts *et al.* 2010; Reiter 2010; World Health Organization 2014). One reason for this statement could be the different feeding behavior of these two vector species. *Ae. aegypti* females are hardly feeding on other hosts than humans (Reiter 2010) and often feed on more than one person within a gonotrophic cycle to gain enough blood for completion (Chow-Shaffer *et al.* 2000; Effler *et al.* 2005). In contrast, *Ae. albopictus* is an opportunistic feeder, taking blood from various mammals such as humans, rabbits, cows, deer, raccoons as well as cats and also from birds (Hawley 1988; Niebylski *et al.* 1994). The differences regarding the feeding pattern may result from different habitat preferences of the two species. *Ae. aegypti* is well-adapted to human environments and is mostly found within 100 m of human dwellings, whereas *Ae. albopictus* is more abundant in suburban and rural areas (Hawley 1988; Gratz 2004; Lambrechts *et al.* 2010; Reiter 2010). Hence, if both species are co-occurring, *Ae. albopictus* has generally less contact to humans and higher contact to other animals (Lambrechts *et al.* 2010). The feeding behavior of *Ae. aegypti* of taking blood almost exclusively from humans and from several persons could lead to a higher number of infected persons by dengue and hence to a larger epidemic disease outbreak compared to *Ae. albopictus* (Effler *et al.* 2005; Richards *et al.* 2006).

The potential to transmit dengue might be lower for *Ae. albopictus* compared to *Ae. aegypti* due to its opportunistic feeding behavior (Richards *et al.* 2006). The lower vectorial capacity rate of the secondary vector *Ae. albopictus* can also be due to its lower infectious rate compared to *Ae. aegypti* (Lambrechts *et al.* 2010). The lower infectious rates of *Ae. albopictus* might result from lower dissemination rates of the virus between midgut and salivary glands within the mosquito (Lambrechts *et al.* 2010). This could reduce the risk for larger dengue outbreaks transmitted by *Ae. albopictus* (Lambrechts *et al.* 2010). Although *Ae. albopictus* shows lower infectious rates and thus lower vectorial capacity rates than *Ae. aegypti*, *Ae. albopictus* is an important vector for DENV and its vectorial capacity should not be underestimated (Vega-Rua *et al.* 2013).

Vega-Rua *et al.* (2013) pointed out that the vector *Ae. albopictus* from France showed a high efficiency in transmitting the chikungunya virus (CHIKV) as well as DENV. Here, *Ae. albopictus* was as successful as the tropical vector *Ae. aegypti*. At an incubation temperature of 28 °C, a percentage of 67 % of *Ae. albopictus* from France were able to deliver DENV after nine days of taking an infectious blood meal, whereas just 21 % of *Ae. aegypti* from Martinique were able to transmit. This study drew attention to the possibility of dengue epidemic outbreaks in Europe transmitted by *Ae. albopictus*. Further, Chan *et al.* (1971) showed already that both mosquito species were the vector of DENV transmission during epidemics of severe dengue in Singapore.

According to a study by Brady *et al.* (2013), *Ae. albopictus* showed optimal survival over a wider temperature range of 20 °C to 30 °C compared to *Ae. aegypti*, which showed optimal survival around a temperature of 21 °C. Moreover, *Ae. aegypti* is not able to overwinter frosty conditions in egg diapause like *Ae. albopictus* (Mitchell 1995). Hence, the distribution range of *Ae. aegypti* is restricted to areas south of the 10 °C cold-month (January) isotherm (Mitchell 1995). Further, *Ae. albopictus* showed the advantage of being able to survive and maintain population growth at higher mosquito density and lower resource availability per individual compared to *Ae. aegypti* (Gratz 2004). This could be an advantage for the breeding possibility in tires with limited space and resources. Especially in the absence of *Ae. aegypti*, *Ae. albopictus* develops in human houses and dwellings and not only in rural and suburban areas (Gratz 2004). *Ae. aegypti* rarely takes natural habitats as breeding sites (Gilotra *et al.* 1967; Gratz 2004).

Thus, on the one hand, *Ae. aegypti* is often declared as primary vector for DENV due to it feeding behavior and higher infectious rate compared to *Ae. albopictus*. On the other hand, *Ae. albopictus* shows an optimal survival at a wider temperature range than *Ae. aegypti* and an ability to establish in the temperate climate in Europe due to overwintering in egg diapause. Moreover, *Ae. albopictus* populations from Europe are as efficient in transmission of CHIKV and DENV as the tropical vector *Ae. aegypti*. *Ae. albopictus* is already established in densely populated European areas, such as areas around Barcelona, Marseille, and Rome. Here, a higher contact to humans can facilitate a dengue outbreak transmitted by *Ae. albopictus* compared to less densely populated areas with similar climatic conditions (Di Luca *et al.* 2001; Roiz *et al.* 2007; Semenza *et al.* 2014). Consequently, *Ae. albopictus* seems to be an efficient vector in Europe and should be considered as possible vector for larger dengue outbreaks in Europe.

1.2.3 Introduction of *Aedes albopictus* to Europe by trade and travel

The introduction of *Ae. albopictus* to Europe has been induced by global trade of goods. The trade of used tires and ornamental plants such as *Lucky Bamboo* (*Dracaena braunii* Engl.) from regions, where *Ae. albopictus* is endemic, to Europe plays an important role (Knudsen 1995; Scholte & Schaffner 2007; Scholte *et al.* 2008). *Ae. albopictus* shows the ability for oviposition in water filled used tires (Hawley 1988; Mitchell 1995). Afterwards, the eggs of *Ae. albopictus* can survive in the used tires, which dried out during storage and transportation, for more than a year. This is due to the eggs' desiccation tolerance. Adults are then able to emerge within a couple of days after rewatering (Knudsen 1995). This ability gives *Ae. albopictus* the opportunity to passively travel over long distances. The import of *Lucky Bamboo* from *Ae. albopictus* infested regions such as southern China introduced *Ae. albopictus* to the Netherlands in 2005 and 2006 (Scholte *et al.* 2008). The survival of the eggs was secured by the transportation and storage of *Lucky Bamboo* on water or gel (Scholte *et al.* 2008). Thus, *Ae. albopictus* could be found in several glasshouses, which stored *Lucky Bamboo* after importation mainly for further shipping within Europe (Scholte *et al.* 2008). As the Netherlands are the main trading point for *Lucky Bamboo* in Europe, the risk of further dispersion of *Ae. albopictus* across Europe was quite high (Scholte *et al.* 2008). According to Linthicum *et al.* (2003), *Ae. albopictus* was introduced to California also via trade of *Dracaena spp.* Vand. ex L. Public travel by planes, ships, and cars is the other main entrance point to non-infested areas (Scholte & Schaffner 2007; Scholte *et al.* 2008; Straetemans 2008). Highways coming from southern Europe, where *Ae. albopictus* is already established, represent such entrance points to other regions in Europe (Straetemans 2008). Along these highways, eggs of *Ae. albopictus* have been found in ovitraps in Germany in 2007 and since 2012, *Ae. albopictus* has been regularly documented (Pluskota *et al.* 2008; Becker *et al.* 2013).

The first introduction and establishment of *Ae. albopictus* in Europe was recorded in Albania in 1979 (Adhami & Reiter 1998). Here, a high abundance of *Ae. albopictus* was found next to used tires. In 1991, the first introduction to Italy was reported (Dalla Pozza & Majori 1992). An establishment in metropolitan France in the Orne and Vienne départements was noted in 1999, which was also induced by trade of used tires (Schaffner *et al.* 2001). In 2000, the first reproduction of *Ae. albopictus* on site was reported in Belgium. Here, *Ae. albopictus* was likely introduced via importation of used tires from Italy, Japan, or the USA (Schaffner *et al.* 2004). In 2004, the first introduction and establishment of *Ae. albopictus* has been reported from Croatia and Spain (Aranda *et al.* 2006; Klobučar *et al.* 2006).

Several projections and studies indicate further establishment of *Ae. albopictus* in Europe due to climate change (Fischer *et al.* 2011; Caminade *et al.* 2012; Fischer *et al.* 2014). The climatic suitability for *Ae. albopictus* was projected to increase in areas, where *Ae. albopictus* is not able to establish yet (Fischer *et al.* 2014). Hence, an increasing climatic suitability can support the establishment of *Ae. albopictus* in western Europe such as Luxembourg, the Netherlands, Belgium, France, and at the Atlantic coastline of the Iberian Peninsula (Fischer *et al.* 2011; Caminade *et al.* 2012). Further risk areas for establishment could be in Central Europe and the United Kingdom (Fischer *et al.* 2011; Caminade *et al.* 2012).

Summarizing, a wide establishment and fast spread of the vector *Ae. albopictus* could be recognized in southern Europe such as in Italy, France, Greece, Spain, and Albania (Straetemans 2008). An increasing abundance of the vector species for DENV can lead to an increased vector-host contact and therefore to an increased risk for dengue transmission (Williams *et al.* 2014). This assumption can be supported by the outbreak of chikungunya (CHIK) with 205 recorded cases in the province of Ravenna, Region Emilia-Romagnain in Italy in 2007 (Rezza *et al.* 2007). Here, CHIKV was probably introduced by an Indian traveler coming from a CHIK endemic area (Angelini *et al.* 2007; Rezza *et al.* 2007). A high abundance of *Ae. albopictus* at that time might have facilitated the outbreak of CHIK (Angelini *et al.* 2007).

1.3 Increasing risk of dengue in Europe

After an establishment of *Ae. albopictus* in Europe, mainly in the Mediterranean (European Centre for Disease Prevention and Control 2009; Thomas *et al.* 2011), the first autochthonous dengue cases could already be reported. In 2010, autochthonous dengue cases could be observed on the Pelješac peninsula and on the isle of Korčula in the south of Croatia (Schmidt-Chanasit *et al.* 2010) as well as in Nice in southern France (La Ruche *et al.* 2010). Regarding the case in Croatia, an elderly person was positively tested for dengue after returning to Germany from a trip to Croatia (Schmidt-Chanasit *et al.* 2010). In France, a person was diagnosed for DENV-1 who did not travel internationally in the recent past, but was visited by friends from the French West Indies (La Ruche *et al.* 2010). Since this person had not received a blood transfusion, he was infected by the vector *Ae. albopictus* in situ. Further autochthonous dengue cases were recorded in Bouches-du-Rhône, southern France in 2013 (Marchand *et al.* 2013) and 2012 in Madeira, Portugal (Wilder-Smith *et al.* 2014). The case in France was very likely infected by DENV-2, which was transmitted via an imported case from Guadelope (Marchand *et al.* 2013). The dengue cases in Madeira differed from the cases on the European mainland, as they were transmitted by the vector *Ae. aegypti* and not by *Ae. albopictus*. After the introduction of *Ae. aegypti* to Europe during the 17–19th centuries, *Ae. aegypti* was extinguished from Europe during the 20th century. Recently, *Ae. aegypti* is again established in Madeira, Portugal and around the Black Sea coast (e.g. Georgia, Russia) (Almeida *et al.* 2007; Schmidt-Chanasit *et al.* 2010; Alves *et al.* 2013; Schaffner *et al.* 2013). For the outbreak of dengue on Madeira with more than 2 000 cases, the virus had its origin very likely in Colombia, Venezuela or Brazil (Wilder-Smith *et al.* 2014). Looking at the several autochthonous cases in Europe, the risk for increasing cases and a dengue outbreak in Europe in times of global change is an important issue and a serious threat, which should be further explored.

1.4 Extrinsic incubation period of dengue

For an increasing risk of dengue fever in Europe, not just the abundance of the vector species, but also the rate of virus amplification is important. Here, the extrinsic incubation period (EIP) and the import of dengue cases from dengue endemic areas have an influence on the potential of dengue transmission in a certain climate and on the speed of dengue transmission. The EIP is defined as "the interval between the acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts" (Houghton Mifflin Company 2015). The EIP of dengue is influenced by the titer of the mosquito-infecting virus dose and strongly influenced by temperature (Watts *et al.* 1987; Thomas *et al.* 2011). Several studies assumed that the higher the temperature is, the lower is the EIP and the higher is the risk for dengue transmission (Focks *et al.* 1995; Sachs & Hedderich 2009; Tjaden *et al.* 2013). According to Watts *et al.* (1987), the EIP lasts around seven days with an air temperature of 32 °C to 35 °C and 12 days and longer with an air temperature lower than 30 °C. Hence, with a longer period of temperatures above 30 °C in parts of Europe, the likelihood of an outbreak and spread of dengue in these areas may increase in times of climate change.

The risk for dengue fever in Europe is increasing, which is indicated by the advancing spread of *Ae. albopictus* and the recent autochthonous dengue cases in Europe. However, to further assess the risk of dengue fever in Europe, it is important to know the relationship between temperature and EIP. Up to now, there exist some, but regarding to the importance of dengue astonishingly few studies that analyzed EIP of dengue at specific temperatures (Blanc & Caminopetros 1930; McLean *et al.* 1974, 1975; Watts *et al.* 1987; Salazar *et al.* 2007; Vega-Rua *et al.* 2013; Xiao *et al.* 2014). Previous approaches to analyze the temperature dependence of the EIP of dengue were conducted by Focks *et al.* (1995) and Tjaden *et al.* (2013). These approaches were based on simple linear models to give first insights and thus might be further improved. Hence, the aim of this study was to analyze the relationship between temperature and EIP in more detail. In addition, future distribution patterns of *Ae. albopictus* and the future duration of the EIP in Europe will be combined to assess potential risk areas for dengue transmission in Europe in times of climate change.

1.5 Hypotheses

Hence, it was hypothesized that

- the linear relationship between EIP and dengue can be optimized applying statistical modeling.
- the length of the EIP will decrease across Europe with advancing climate change, so that areas with a shorter EIP will increase and can be expected further north. In addition, the areas where the EIP is longer than the life expectancy of the mosquito will decrease.
- the climatic suitability for dengue transmission will increase across Europe due to an increasing climatic suitability for the vector mosquito *Ae. albopictus* and a decreasing EIP with advancing climate change. Thus, areas with higher climatic suitability for dengue transmission will increase and can be expected further north.

2 Methods

2.1 Study area

2.1.1 Current climate

The designated study area for this thesis is Europe (26.0°N to 76.0°N and 26.0°E to 44.8°W) which is characterized by a climatic gradient from oceanic in Western Europe to continental climate in Eastern Europe (temperature differences between warmest and coldest month; Fig. A1). The climate data was derived from WorldClim (`http://worldclim.org`, Hijmans *et al.* 2005). Annual mean temperatures range from -12.3 °C to 24.5 °C and temperatures decrease from south-west to north-east. Exceptions are the Alps in Austria, Switzerland, northern Italy, and south-east France, the Pyrenees between France and Spain, and the Carpathians across the eastern European member states with lower temperatures caused by high elevation.

The mean temperature of the warmest month ranges from -2.0 °C to 34.8 °C. Mean temperatures of the warmest month generally decrease from south to north independently of continentality. Higher elevated areas such as in the Cantabrian Mountains in northern Spain, the Massif Central in France, the upland areas in Germany, the Dinaric Alps in south-east Europe, the Alps, the Pyrenees, and the Carpathians show a lower mean temperature of the warmest month. Further, Great Britain and Ireland are characterized by lower mean temperatures of the warmest month due to a oceanic climatic influence.

The mean temperature of the coldest month ranges from -21.5 °C to 16.7 °C. The pattern is similar compared to the annual mean temperatures. Mean temperatures of the coldest month decrease also from south-west to north-east with the exceptions of the Alps, Pyrenees, and Carpathians. However, these patterns are less pronounced compared to the annual mean temperatures. Great Britain and Ireland show higher mean temperatures of the coldest month due to oceanic climatic influence.

Annual precipitation ranges from 10 mm a^{-1} to 2852 mm a^{-1}. Low annual precipitation patterns can be found in the center and south of the Iberian Peninsula, Eastern Germany, Poland, Hungary, east of Greece and Macedonia, central Turkey, south-east of Romania, and at the northern coastline of the Black Sea as well as in the north of Sweden and Finland. High annual precipitation can be found at the north-western coastline of the North Atlantic Ocean, Norwegian Sea, Mediterranean Sea, and Adriatic Sea as well as in mountain regions such as the Alps, the Pyrenees and the Carpathians.

2.1.2 Projected future climate

Looking at the projected climate parameters annual mean temperature, mean temperature of the coldest and of the warmest month as well as annual precipitation, a general trend can be seen for both of the new IPCC5 scenarios RCP4.5 ($4.5\,W\,m^{-2}$ in 2100) and RCP8.5 ($8.5\,W\,m^{-2}$ in 2100) and for the two time steps 2041–2060 (2050s) and 2061–2080 (2070s). According to the Fifth Assessment Report of IPCC, the RCP refer to the total radiative forcing in year 2100 compared to 1750 (IPCC 2013). Data on land use change and anthropogenic emission of air pollutants are integrated in each scenario. The RCP4.5 represents a stabilization scenario with stabilization by 2100. The RCP8.5 describes a scenario with very high greenhouse gas emissions and does not reach its radiative forcing peak by 2100. Thus, the general trend regarding the projected climate parameters is more distinctive in the scenario RCP8.5 than in RCP4.5 and in the later time period than in the earlier time period (Fig. A2–Fig. A5), respectively.

Further IPCC5 scenarios are RCP2.6 ($2.6\,W\,m^{-2}$ in 2100) and RCP6.0 ($6.0\,W\,m^{-2}$ in 2100), whereas RCP2.6 refers to a very low radiative forcing level and is a peak and decline scenario (Van Vuuren *et al.* 2011; IPCC 2013). Thus, the scenario RCP2.6 seems to be unlikely to be achieved under the current greenhouse gas emissions, as these would need to be substantially reduced (Van Vuuren *et al.* 2011). Hence, a scenario between RCP4.5 and RCP8.5 for climate change projections is likely to become real. Further, since dengue endangers public health, it is important to analyze the worst case scenario applying climate change projections based on the scenario RCP8.5, which represents the highest radiative forcing. Hence, the scenarios RCP4.5 and RCP8.5 were the chosen climate change projections in this study. The climate projections were derived from WorldClim (`http://worldclim.org`). The projections are based on the most recent GCMs used in the Fifth Assessment Report of IPCC (IPCC 2013) and are downscaled to 5 arcminutes. Here, the model is driven by the earth system model MPI-ESM-LR (Max-Planck-Institut für Meteorologie 2012; for more details: special section in the Journal of Advances in Modeling Earth Systems `http://onlinelibrary.wiley.com/journal/10.1002/%28ISSN%291942-2466/specialsection/MPIESM1`).

The projected annual mean temperatures show a trend of greater warming from south-west to north-east with a higher increase in the north-east of Europe (Fig. A2–Fig. A5) in the two time steps 2050s and 2070s. An exception is the center of the Iberian Peninsula, which shows a higher annual mean temperature increase in the future compared to the rest of Western Europe. The projected mean temperatures of the warmest month show a higher increase from the north-east to the south-west of Europe. Hence, the upper tip of Spain shows a lower temperature increase compared to the same longitude in the east of Europe. The projected mean temperatures of the coldest month show a temperature increase from south-west to north-east. Regarding the projected annual precipitation, a decrease of precipitation in the south of Europe and an increase in the north can be expected in the future.

Summing up, current areas with low mean annual temperatures and low mean temperatures of the coldest month in the north-east of Europe show a higher increase in temperature compared to the south-west of Europe. An exception is the center of the Iberian Peninsula which also shows a higher temperature increase. The highest temperature increase in the warmest month can be expected in the south of Europe. Areas with already low annual precipitation such as the center and south of the Iberian Peninsula, Hungary, and the east of Greece and Macedonia are projected to get drier in the future.

The spatial patterns of temperature and precipitation and the projected climate in Europe were provided, as these climatic variables influence the EIP and the climatic suitability for *Ae. albopictus*. For the climatic suitability of *Ae. albopictus*, the annual mean temperature, the temperature of the warmest, and the temperature of the coldest month play an important role. The temperature of the warmest month is also important for the EIP, as the EIP becomes shorter with increasing temperature. The climatic variable annual precipitation was chosen to get an impression of the changing precipitation patterns due to climate change in Europe. Changing precipitation patterns might influence the climatic suitability for *Ae. albopictus* and consequently the risk of dengue fever in Europe.

2.2 Temperature dependence of the EIP of dengue

2.2.1 Literature review on the relationship between EIP and temperature

To analyze the relation between temperature and EIP, the critically reviewed literature on the EIP of dengue by Tjaden *et al.* (2013) was extended with newer literature from 2013 onwards (Tab. A1). Experimental studies about the vector *Ae. albopictus* of dengue are still widely missing except for two recent studies by Vega-Rua *et al.* (2013) and Xiao *et al.* (2014). Therefore, mainly studies on the vector *Ae. aegypti* could be reviewed. Both mosquitoes are vector species of dengue. *Ae. aegypti* is established in Madeira, Portugal (Almeida *et al.* 2007; Schmidt-Chanasit *et al.* 2010; Alves *et al.* 2013), whereas *Ae. albopictus* is already established on the European mainland (European Centre for Disease Prevention and Control 2009; Thomas *et al.* 2011). Only nine experimental studies with all in all 66 observations of the EIP at different temperatures could be found. Therefore, the studies addressing either *Ae. aegypti* or *Ae. albopictus* as vector were analyzed together and not separately. These pre-selected studies varied in the experimental settings regarding provenances and sources of the mosquitoes (e.g. captured or raised in lab), genotypes or strains of DENV, type of injection and further experimental measurements (Tab. A1). With one exception, all experimental studies analyzed the EIP of DENV type 2.

For the purpose of this study, the experimental studies were especially screened for the type of infection (feeding, intrathoracic injection) and for type of transmission. Regarding the type of infection, an infection by intrathoracic injection shortens the EIP due to a circumvention of the midgut infection and escape barrier (Smith *et al.* 2005). To avoid an underestimation of the EIP not an intrathoracic injection, but an oral infection via feeding on viraemic vertebrates or artificial blood meals was suggested by Tjaden *et al.* (2013). Applying an oral infection represents a more natural infection scenario. Regarding the ability of transmission, it is important to test an infection of the salivary glands or an actual transmission to a host by biting vertebrates. Testing complete heads or bodies of the mosquitoes is not appropriate for the current study, as the midgut escape barrier and the salivary gland infection barrier would be neglected (Black *et al.* 2002). The midgut and salivary gland infection barriers as well as the midgut and salivary gland escape barriers have to be overcome by the virus for replication in organs and tissues of the mosquito and a final transmission to a host (Black *et al.* 2002; Smith *et al.* 2005).

In summary, the EIP data based on experiments with realistic transmission scenarios were further used in this study. Realistic transmission scenarios were selected based on the following conditions: feeding as type of injection and checked salivary glands or confirmed transmission by biting a vertebrate as analysis of the potential of transmission by the mosquito.

2.2.2 Modeling the relationship between EIP and temperature

For modeling the relationship between EIP and temperature, literature research was applied to find the most appropriate model. Approaches such as survival analysis or general linear model (GLM) fitting were considered and tested. Different distributions were applied to consider different statistical distributions underlying the ecological data in the current study. Applying the GLM as a statistical standard method, different statistical distributions such as gamma, gaussian, and inverse gaussian distribution were used to reveal the best model (Fig. A6). Here, the problem was that the EIP was not measured from the beginning of the experiments, but at certain temperatures. Hence, it was not possible to know, whether the virus could already have been transmitted before the measures started at the certain temperature. Therefore, survival analysis was tested as an advanced alternative taking into account that the exact onset of the ability of dengue transmission was not analyzed in the reviewed experimental studies (Sachs & Hedderich 2009). This approach was also applied by Chan & Johansson (2012) to analyze the extrinsic and intrinsic incubation periods of DENV. In the current study, the weibull, gaussian, lognormal, loglogistic, and exponential distributions were tested in different models of the survival analysis (Fig. A7). To find the best model, the Akaike Information Criterion of the models with different distributions was analyzed. When comparing the models, the lower AIC value refers to a higher probability of assuming the right distribution. This was done for the GLM and survival analysis separately, as two different approaches can not be compared.

Both, the GLM approach and the survival analysis assume a linear model. This means, the higher the temperature is, the shorter is the EIP. Hence, physiological limitations regarding high temperatures were not considered. Therefore, a nonlinear model as a third model was applied for analysing the temperature dependence of the EIP rate, as it has been done in several studies to take the influence of temperature on biological processes into account (Briere *et al.* 1999; Paaijmans *et al.* 2009; Mordecai *et al.* 2013; Johnson *et al.* 2015). In the studies by Paaijmans *et al.* (2009), Mordecai *et al.* (2013), and Johnson *et al.* (2015), the non-linear unimodal function according to Briere *et al.* (1999) was used to analyze the relationship between EIP rate of malaria and temperature. This approach was also chosen in the current study to analyze the unimodal temperature dependence of the EIP rate of dengue. Since *Aedes* mosquitoes are the vector of dengue, temperature effects on biological processes within the mosquitoes have to be considered. Physiological processes and traits in the ectotherm vector mosquitoes are constrained by low and high temperatures as well (Huey & Stevenson 1979; Angilletta 2009; Dell *et al.* 2011; Amarasekare & Savage 2012). Thus, the temperature dependence of biological processes is influencing the relationship between EIP of dengue and temperature, although dengue is a virus and not a parasite like malaria. By applying the non-linear unimodal function, the slowdown of biological processes by both too low and too high temperatures is taken into account.

The left-skewed unimodal function according to Briere *et al.* (1999) consists of the temperature T as predictor and the three parameters minimum temperature T_0, maximum temperature T_m and a rate constant c, so that $EIP_{rate} = c \times T \times (T - T_0) \times (T_m - T)^{1/2}$ (Mordecai *et al.* 2013 defined by Briere *et al.* 1999). Exact data on the minimum and maximum temperatures of virus development are yet missing. The estimated maximum temperature of 40 °C was chosen according to Brady *et al.* (2013), because 40 °C is the upper temperature limit for the survival of *Ae. albopictus.* Consequently, DENV will not be acquired by the vector *Ae. albopictus* above 40 °C. For the minimum temperature, most accurate values are needed, as the study area is located in the temperate climate zone. Hence, the EIP at low temperatures plays an important role in modeling the risk of dengue fever in Europe. To model the best fitting minimum temperature, temperatures between 4 °C and 12 °C (1 °C steps) were considered as minimum temperature in the function according to Briere *et al.* (1999). The lower temperature limit of 4 °C indicates the lowest mean temperature requirement for the survival of *Ae. albopictus* (Brady *et al.* 2013). The upper temperature limit of 12 °C was taken, as 13 °C is the lowest temperature confirmed for dengue transmission in the reviewed experimental studies analysing the EIP of dengue. To optimize the non-linear function regarding the minimum temperature, the residual standard errors (RSE) were compared. Setting the minimum temperature to $T_m = 9$ °C resulted in the smallest RSE and was therefore used for the final model (Fig. 2). The non-linear unimodal models were fitted applying nonlinear least squares regression as applied by Mordecai *et al.* (2013).

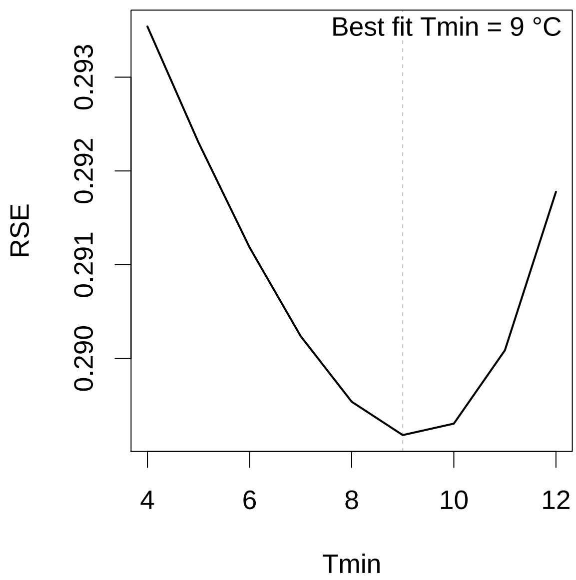

Fig. 2: *Residual standard errors of the optimization of the EIP model regarding the minimum temperature T_m. To model the best fitted minimum temperature, temperatures between 4* °C *and 12* °C *(in 1* °C *steps) were tested applying the left-skewed unimodal function according to Briere* et al. *(1999). The minimum temperature of $T_m = 9$* °C *resulted in the lowest RSE and was thus further used for the final model of the EIP.*

2.3 Combined assessment of the EIP of dengue and the climatic suitability for *Aedes albopictus*

To project the climatic suitability for Dengue transmission, the first step was to project the EIP durations across Europe. For this step, the modeled EIP data in the previous section was transferred to the changing climate in Europe. This was cartographically visualized. Afterwards, the transferred EIP was combined with the climatic suitability of *Ae. albopictus* by multiplication. Finally, the risk for dengue transmission in Europe could be presented in maps.

2.3.1 Climate data

In general, for climate impact studies, such as studies regarding the development of vector-borne diseases, climate models on regional rather than on global spatial scales are more appropriate as air temperature and precipitation patterns vary regionally (Giorgi & Diffenbaugh 2008; Jacob 2008). Regional models take effects of topography, coastlines and further landscape features on temperature and precipitation patterns more into account than global models (Giorgi & Diffenbaugh 2008; Jacob 2008).

In this study, future projections on the climatic suitability for dengue transmission across Europe are based on data provided by the World Climate Research Program Coordinated Regional Downscaling Experiment (EURO-CORDEX) initiative. In particular, the projected climate data are based on the regional model CCLM4-8-17, which is a nonhydrostatic regional climate model generated from the local model of the German Meteorological Service by the CLM-Community (`http://clm-community.eu`). This regional climate model is the current recommended version by the CLM-Community. The regional model is driven by the earth system model MPI-ESM-LR and is dynamically downscaled for Europe (Max-Planck-Institut für Meteorologie 2012; for more details: special section in the Journal of Advances in Modeling Earth Systems `http://onlinelibrary.wiley.com/journal/10.1002/%28ISSN%291942-2466/specialsection/MPIESM1`, EURO-CORDEX2015). The MPI-ESM-LR model exhibits some improvements compared to the previous model ECHAM5/-MPIOM such as new and improved representation of aerosols, surface albedo, and short-wave radiation transmission (Max-Planck-Institut für Meteorologie 2012). Further changes were e.g. the incorporation of the land surface with interactive vegetation dynamics and of the coupled carbon cycle (Max-Planck-Institut für Meteorologie 2012).

The regional climate change patterns were projected based on the new IPCC5 emission scenarios RCP4.5 and RCP8.5 and were separately analyzed for the three time periods 2021–2040 (2030s), 2041–2060 (2050s), and 2061–2080 (2070s) (IPCC 2013; EURO-CORDEX 2015). The EURO-Cordex simulation with a spatial resolution of 0.11 deg (EUR-11) makes a small-scaled analysis of the climatic suitability for dengue transmission in Europe possible. For both scenarios, daily temperatures were averaged over 20 years for each of the three time periods to reduce statistical noise and natural variability as applied by Thomas *et al.* (2011).

2.3.2 Projected EIP across Europe

For the projection of EIP durations across Europe, raster files with information about the maximum number of consecutive days of the temperature from $x = 9\,°C, \ldots, 40\,°C$ (1 °C steps) were prepared in cooperation with Nils Tjaden (Department of Biogeography, University of Bayreuth). The temperature range resulted from the previously modeled EIP (1 °C steps, see section 2.2.2). A selection algorithm was generated which started a selection process on the 1st of January. The algorithm then tested, whether the temperature on this day was higher or equal than x. If yes, it tested how long the temperature stayed above or equal x. This procedure was repeated for every day of the year, starting at the 1st of January and ending at the 31th of December. Only the longest periods of consecutive days were retained. This process was applied for the two scenarios RCP4.5 and RCP8.5 and the time periods 2030s, 2050s, and 2070s separately.

Consecutively, the raster files providing the longest period of the temperature x were used to analyze, whether the period of the temperature x is long enough for the virus amplification. This process started with the raster file regarding the temperature of 9 °C and ended with the raster file regarding the temperature of 40 °C. The higher the temperature was, the lower was the required number of consecutive days with this temperature. Raster cells in which the temperature requirement of a low temperature was fulfilled, were replaced with the next highest fulfilled temperature until no higher temperature was fulfilled any more. Afterwards, each raster cell provided information about the highest temperature for which the length of consecutive days was long enough. This means, the higher the fulfilled temperature was, the shorter was the EIP and the higher will be the risk for dengue transmission. The information about the fulfilled temperature was translated into the respective length of EIP. The whole process was applied for the two scenarios and three time steps separately. The six resulting raster files with a resolution of 5 arcminutes were cartographically visualized. Mapping the EIP, the classes of EIP length refer to their 20 % quantiles. An EIP length of more than 60 days was marked, as the EIP then would exceed the life expectancy of the mosquito *Ae. albopictus* (Brady *et al.* 2013). The borders of Europe are drawn according to Sandvik (2009).

2.3.3 Projected climatic suitability for dengue transmission

To project the climatic suitability for dengue transmission in Europe, as well the EIP length as the climatic suitability for *Ae. albopictus* plays an important role. Both the requirements for a relatively short EIP and the occurrence of the vector are needed to get a high risk for successful transmission. The raster files with information about the climatic suitability for *Ae. albopictus* were prepared in cooperation with Dr Anja Jaeschke (Department of Biogeography, University of Bayreuth). An ensemble modeling approach (R package “biomod”) was applied for the distribution model. The used bioclimatic variables were annual mean temperature (BIO1), maximum temperature of warmest month (BIO5), minimum temperature of coldest month (BIO6) as well as mean temperature of the driest (BIO9), the warmest(BIO10), and the coldest (BIO11) quarter. These variables were downloaded from WorldClim (`http://worldclim.org`). The climatic parameters were statistically selected by applying the method of hierarchical partitioning, which analyzes the share of variance of the climatic parameters explaining the current distribution of *Ae. albopictus*. The potential future distribution of *Ae. albopictus* was projected for the scenarios RCP4.5 and RCP8.5 and for the time periods 2021-40 (2030s), 2041-60 (2050s) and 2061-80 (2070s). The projections are based on the earth system model MPI-ESM-LR (Max-Planck-Institut für Meteorologie 2012). The climatic suitability for *Ae. albopictus* ranged from 0 to 1. The classes were built in probability steps of 0.1. The step from 0.6 to 0.8 was considered as high climatic suitability and hence was summarized in one class. No values above 0.8 were yielded.

The climatic suitability for dengue transmission across Europe was projected by combining (multiplying) the climatic suitability for *Ae. albopictus* and the previously modeled EIP. For this procedure, the EIP data was first inversed and then scaled from 1 (low EIP) to 0 (high EIP). Thus, low EIP data could be interpreted as low risk and a multiplication with the climatic suitability for *Ae. albopictus* was possible, as there low values also indicate a low risk for dengue transmission. The resulting climatic suitability for dengue transmission for the scenarios RCP4.5 and RCP8.5 and for the time periods 2030s, 2050s and 2070s was cartographically visualized. Classes were built in probability steps of 0.1 up to 0.5. No values above 0.7 were yielded. The values above 0.5 were considered as high risk for transmission and thus were summarized in one class. EIP values (days) higher than 60 were marked, as the length of EIP would exceed the life expectancy of *Ae. albopictus*. Hence, the risk for transmission of dengue would be very low, if it exists at all.

All analyses in this study were conducted in R 3.0.2 (R Core Team 2013) using the packages “raster” (Hijmans 2015), “rgdal” (Bivand *et al.* 2014), “sp” (Pebesma & Bivand 2005), “survival” (Therneau 2014), and “vegan” (Oksanen *et al.* 2013) and in QGis 2.4.0.

3 Results

3.1 Relationship between EIP of dengue and temperature

Temperature dependence of EIP of dengue can be modified by the virus load and by the way it is taken up experimentally. A realistic scenario of transmission (feeding and not intrathoracic injection as type of infection and infection of salivary glands of mosquitoes or confirmed virus transmission) was provided by 38 observations in eight different experimental studies. The experimental studies differed in their settings regarding the virus amount for the infection of the mosquitoes and regarding the method testing a potential transmission via infected salivary glands (uptake of DENV by salivary glands) of mosquitoes or confirmed virus transmission (Fig. 3). The transmission of DENV (e.g. transmission of the virus by the mosquito to monkeys or mice) was confirmed in 14 observations. Infection of salivary glands of the mosquitoes *Ae. aegypti* and *Ae. albopictus* was confirmed in 36 observations. Both criteria for potential transmission overlapped in 12 observations. Thus, all in all, 38 observations could be used in this study to model the relationship between EIP of dengue and temperature. Generally, simulating a potential transmission via infected salivary glands lead to a shorter EIP compared to simulating an actual transmission to a host (Fig. 3 b).

The modeled virus activity (EIP rate) in dependence of temperature was restricted to the temperature range between 9 °C and 40 °C (Fig. 4). Above 9 °C, the EIP rate (1/days) increased slowly in the beginning. Then, the EIP rate increased faster and nearly linear between 15 °C and 30 °C. The maximum of the function (EIP rate = 0.17) was reached at a temperature of $T = 33$ °C. For higher temperatures, the EIP rate steeply decreased until the maximum temperature of 40 °C with an EIP rate of 0 was achieved.

Regarding the relationship between EIP (inverse EIP rate; days) and temperature, the EIP decreased exponentially with increasing temperature for temperatures lower than 33 °C (Fig. 5). The EIP increased again for higher temperatures. Regarding the five EIP data points from experimental studies related to the vector *Ae. albopictus*, the EIP durations matched the modeled EIP curve very good. The differences between measured and modeled EIP were between 0 days ($T = 26$ °C) and 3 days ($T = 28$ °C). All modeled EIP, except the EIP at 28 °C, were lower compared to the experimentally measured EIP.

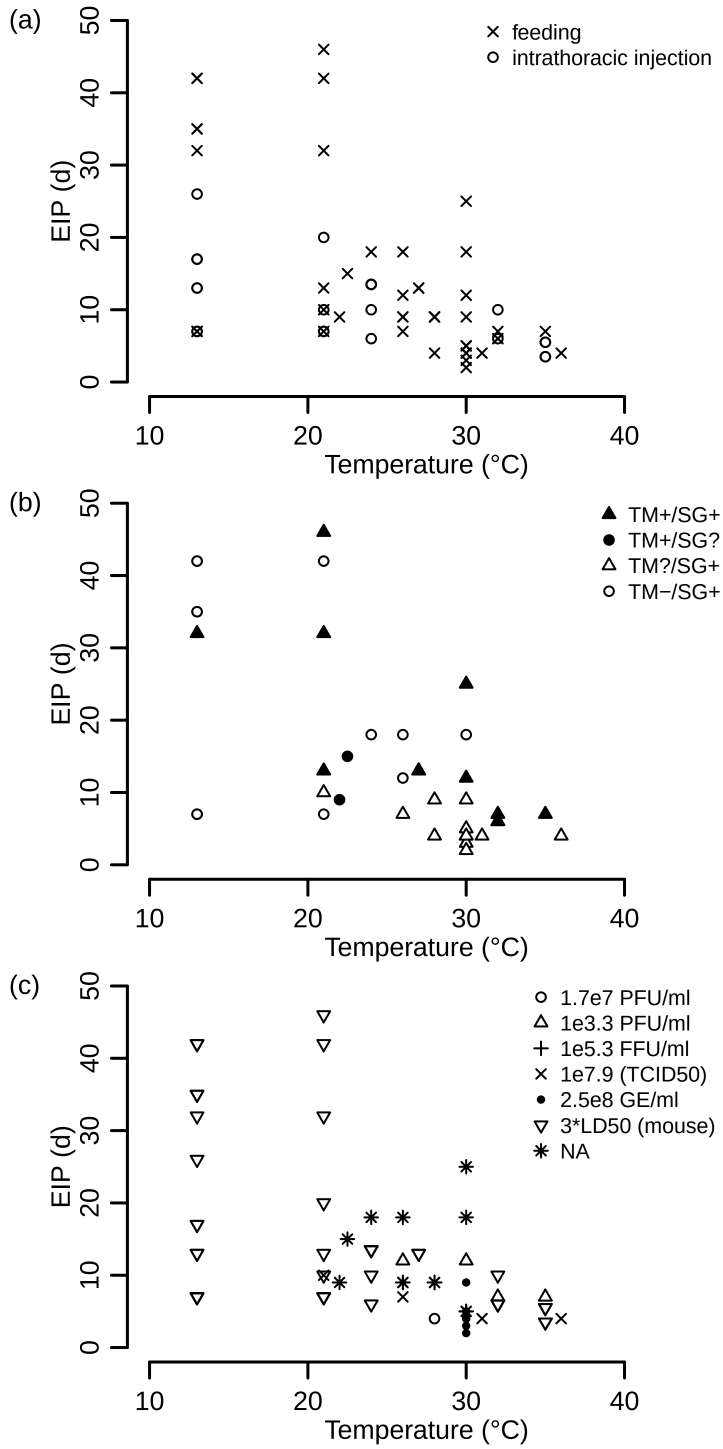

Fig. 3: *Reviewed data on the EIP of dengue and temperature. Each point gives the time interval between infection of the vector mosquito Ae. aegypti or Ae. albopictus and a potential transmission of dengue by the vector (N = 38). (a) illustrates the different methods used for uptake of DENV by the vector. DENV was taken up by feeding on infected animals or artificial blood meals containing the virus or by intrathoracic injection. (b) compares different method combinations for simulating a potential transmission. Combination of methods could be built by confirmed transmission (TM+), non-proven transmission (TM?), rejected transmission (TM-) and confirmed infection of salivary glands (SG+) as well as non-proven salivary glands (SG?). (c) gives an overview of the different used amount of virus within the dataset of infected mosquitoes by feeding. PFU: plaque forming units;* $TCID_{50}$*: median tissue culture infective dose; GE: genome equivalents;* LD_{50}*: mean lethal dose.*

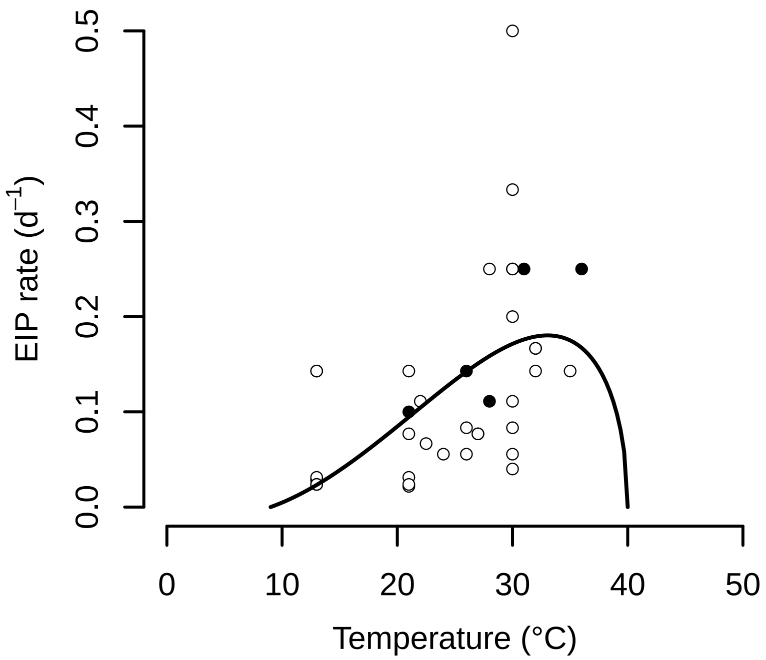

Fig. 4: *Modeled relationship between EIP rate (1/days) of dengue and temperature. The left-skewed unimodal function according to Briere* et al. *(1999) was applied. The margins of the function were 9*°C *and 40*°C. *Each point gives the time interval between infection of the vector mosquito and potential transmission of dengue by the vector (N = 38). White points refer to Ae. aegypti. Black points refer to Ae. albopictus.*

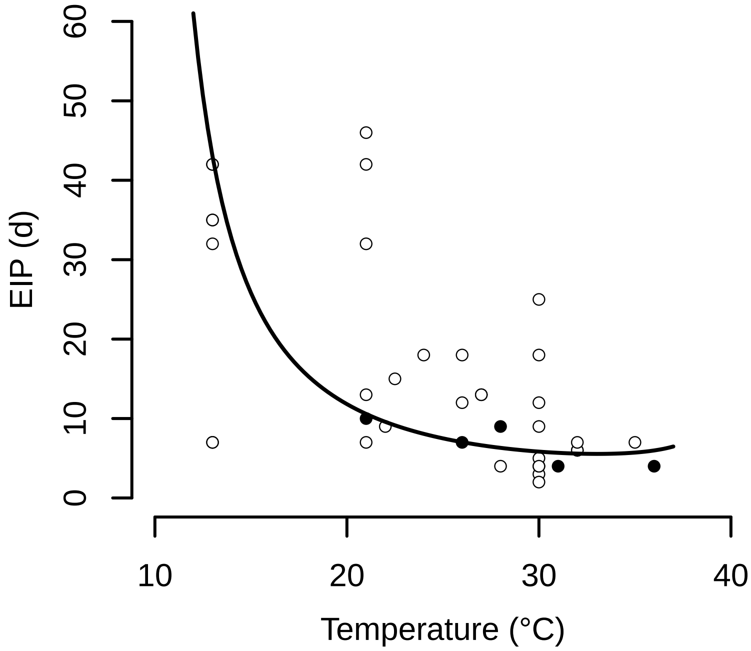

Fig. 5: *Modeled relationship between EIP (days) of dengue and temperature. The left-skewed unimodal function according to Briere* et al. *(1999) was applied. The margins of the function were 9*°C *and 40*°C. *Each point gives the time interval between infection of the vector mosquito and potential transmission of dengue by the vector (N = 38). White points refer to Ae. aegypti. Black points refer to Ae. albopictus.*

3.2 Projected trends and patterns of EIP across Europe

The EIP of dengue across Europe was modeled based on the IPCC5 emission scenarios RCP4.5 and RCP8.5 for the three time periods 2021–2040 (2030s), 2041–2060 (2050s), and 2061–2080 (2070s) (Fig. 6). The temperature requirements for the EIP were based on the previously analyzed relationship between EIP and temperature. At most locations across Europe, the EIP was projected to become shorter for both scenarios in the future. Comparing time periods and scenarios, the EIP is expected to shorten most distinctively in the more distant future, especially in the scenario RCP8.5 compared to the scenario RCP4.5. Hence, the highest share of areas with a shortened EIP and therefore increased risk for virus amplification were projected for the 2070s based on the scenario RCP8.5. Regarding the spatial trend, areas with a short EIP are expected to expand with advancing climate change. Within each scenario and time step, the modeled EIP duration was longer in the north than in the south of Europe. Mountainous regions showed a longer EIP duration than surrounding areas also in the South.

Strong spatial gradients could be detected for EIP durations across Europe. The valleys of the rivers Guadiana and Gualdalquivir, the Andalusian Plain and parts of the south-western and southern coastline of the Iberian Peninsula are expected to show an EIP of 5 to 7 days in all observed time periods and both scenarios. The total European area that exhibits EIP of 5 to 6 days is found to be larger considering the RCP8.5 scenario compared to the scenario RCP4.5. This pattern was especially detected for the 2050s and 2070s. An EIP between 5 and 6 days is the shortest projected EIP in Europe until 2080 and was only projected for the south-western part of the Iberian Peninsula. Besides the Iberian Peninsula, larger areas with an EIP of 6 to 7 days were detected for the south-eastern parts of Europe for the 2050s and 2070s (Danube valley along the border of Bulgaria and Romania, and parts of Greece as well as Turkey along the Aegean coastline, parts of Bulgaria and Russia). Regarding the scenario RCP4.5, the areas of a short EIP in the south-eastern parts of Europe were projected to decrease again after the 2050s. Considering the scenario RCP8.5, these areas are still expected to increase after the 2050s. In addition, areas with an EIP of 6 to 7 days that lie in the Po valley as well as at the south-eastern tip of Italy and on the Italian islands Sardinia and Sicily were detected to expand in the more distant future (2050s and 2070s) considering the scenario RCP8.5. In the 2070s of the scenario RCP8.5, the area with an EIP of 6 to 7 in South-west Russia was projected to strongly expand.

Distinct temporal shifts regarding EIP durations and expansion of areas with shortened EIP could be detected across Europe. First areas in France with an EIP of 7 to 11 days were projected for parts north of the Pyrenees in the 2050s considering the scenario RCP4.5. This area was detected to increase in the 2070s. Looking at the scenario RCP8.5, this area is expected to be larger in the 2030s and the consecutive expansion of this area was detected to be wider. In Germany, the shortest EIP of 11 to 21 days is expected to be first observed in the Rhine rift valley and in some fragmented parts in the eastern part in the 2030s (RCP4.5). These projections increased further into the future and new areas in the north-western, north-eastern and south-eastern part were projected to occur in the 2050s and 2070s (RCP4.5) and in the 2030s and 2050s (RCP8.5), respectively. In the 2070s (RCP8.5), nearly the whole of Germany was detected to experience an EIP of 11 to 21 days. The upland areas such as the Bavarian Forest, Ore Mountains (*Erzgebirge*), and the Rhenish Slate Mountains (*Rheinisches Schiefergebirge*) were exceptions. In the 2070s (RCP8.5), these upland areas were projected to show a long EIP of 21 to 60 days. Most of Great Britain is expected to show an EIP of 21 to 60 days for all observed time periods and both scenarios, besides the 2070s of the scenario RCP8.5. For the southern tip of Great Britain, areas with an EIP of 11 to 21 days were projected for the time period 2070s of the scenario RCP4.5. These areas were projected to increase in the 2050s (RCP8.5). In the 2070s (RCP8.5), about the half of Great Britain is expected to be characterized by an EIP of 11 to 21 days.

As a consequence of elevational decrease of temperature, mountainous regions are expected to show a longer EIP compared to surrounding areas (all time periods and scenarios). Especially concerned are mountainous regions such as the Cantabrian Mountains, Massif Central, the Appennines, Dinaric Alps, and Carpathian Mountains. Planes, river valleys such as Danube, Guadiana as well as Gualdalquivir, and larger water surfaces such as Lake Ladoga as well as Lake Vänern (2070s, RCP8.5) tend to show shorter EIPs compared to surrounding areas. Small areas with a shorter EIP of 11 to 21 days were projected for northern Europe around larger water surfaces compared to the surrounding area with an EIP between 21 and 60 days (all time periods and scenarios).

An EIP of more than 60 days was modeled for several European areas. This is expected to be true for Iceland, the northern part of Great Britain, the north-western part of Scandinavia (especially in the Scandinavian Highlands), the Alps, and the Caucasus Mountains. In these regions, the length of the EIP is likely to exceed the life expectancy of the vector mosquito *Ae. albopictus* in all three time periods and for both scenarios. Except for Iceland, the areas in all of these regions were projected to decrease over time. The decrease is likely to be stronger for the RCP8.5 scenario than for the RCP4.5 scenario. The Pyrenees and parts of the Carpathian Mountains were projected to show an EIP of more than 60 days in all three time periods of the scenario RCP4.5 and the time period 2030s of the scenario RCP8.5.

In summary, areas of a high EIP were detected to be in mountainous regions and northern parts of Europe such as Ireland, northern parts of Great Britain, and Scandinavia. Areas characterized by a short EIP were projected for the south-western part of the Iberian Peninsula, for south-eastern parts of Europe such as the Danube valley, parts of Greece and Turkey, for parts of Italy and south-western parts of Russia.

3.3 Projected climatic suitability for *Aedes albopictus*

The climatic suitability for *Ae. albopictus* was modeled across Europe for the time steps 2021–2040 (2030s), 2041–2060 (2050s), and 2061–2080 (2070s) based on the IPCC5 scenarios RCP4.5 and RCP8.5 (Fig. 7). In general, the climatic suitability for *Ae. albopictus* was projected to increase with advancing climate change. Within each scenario and time step, the modeled climatic suitability for *Ae. albopictus* was generally higher in the south than in the north. Exceptions were mountainous regions, the south-western part of Russia and the Iberian Peninsula. The detected trends were generally stronger for the scenario RCP8.5 compared to RCP4.5.

Strong spatial gradients of the climatic suitability for *Ae. albopictus* were projected across Europe. In the valleys of the rivers Guadiana and Gualdalquivir, the Andalusian Plain and central parts of the Iberian Peninsula, the climatic suitability for *Ae. albopictus* is expected to decrease (values from 0.0 to 0.2) with advancing climate change. The decrease was found be more pronounced for the scenario RCP8.5 than for the scenario RCP4.5. Here, the largest area with very low

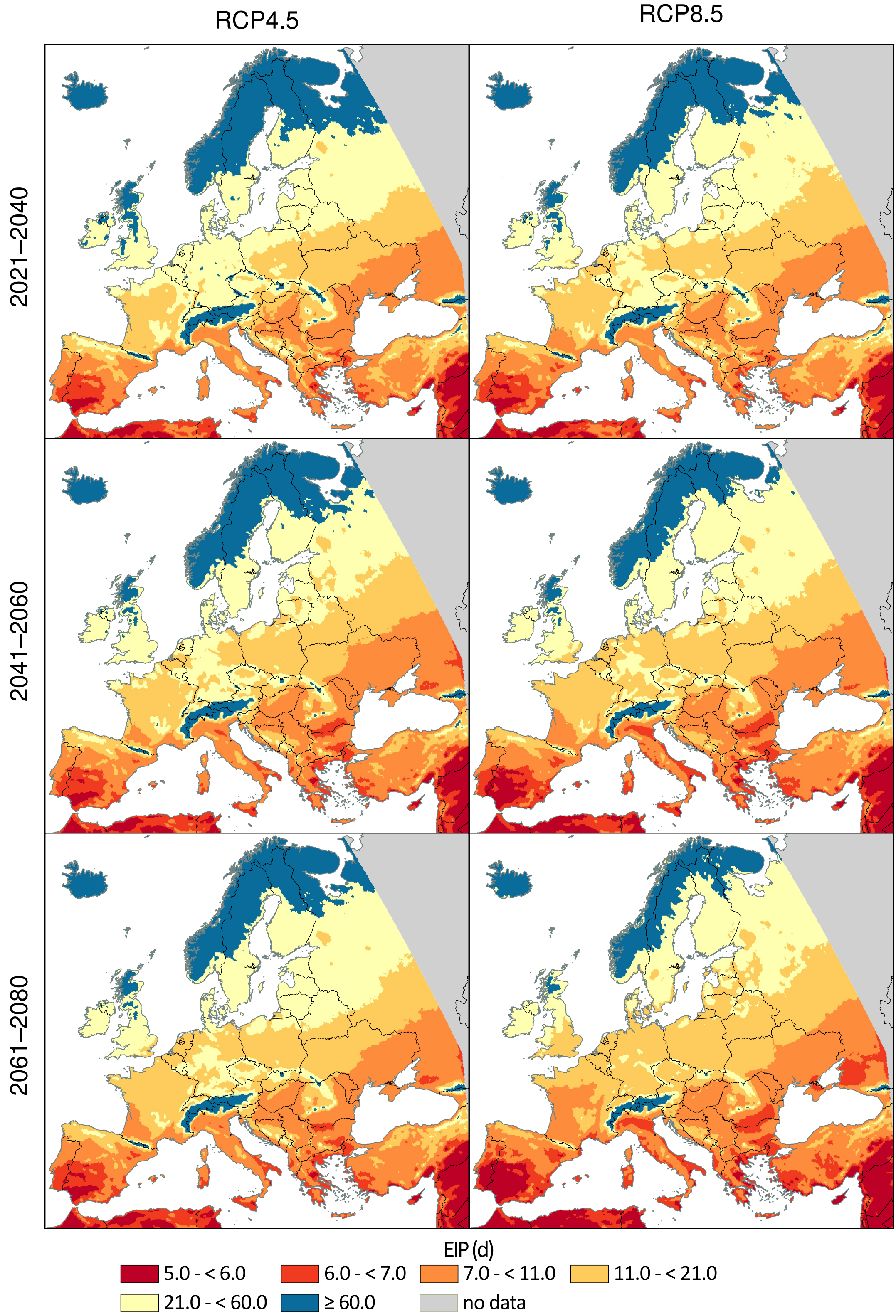

Fig. 6: *Modeled EIP (days) across Europe during the 21st century based on two divergent IPCC5 scenarios (RCP4.5 and RCP8.5). The classes of EIP length refer to their 20 % quantiles. An EIP length of more than 60 days was marked in blue, as the EIP then would exceed the life expectancy of the mosquito Ae. albopictus.*

climatic suitability (values between 0.0 and 0.1) was projected for the 2070s (RCP8.5). For the south western part of Russia, a low climatic suitability (values between 0.1 and 0.4) was projected for the scenario RCP4.5, whereas the climatic suitability was projected to decrease in further future. The lowest climatic suitability of 0.0 to 0.1 in this region was detected for the 2070s (RCP8.5).

The mountainous regions are expected to be generally characterized by a lower climatic suitability for *Ae. albopictus* compared to the surrounding areas (all time periods and scenarios). The concerned mountainous regions would be the Cantabrian Mountains, Massif Central in France, Pyrenees, Dinaric Alps, Carpathian Mountains, Alps, and Caucasus Mountains. Areas along the coastline of the Iberian Peninsula, in southern as well as south-western parts of France, Italy and along the coastline of the Black Sea as well as along the Adriatic coastline were projected to be characterized by a high climatic suitability for *Ae. albopictus* (values between 0.5 and 0.8). Along the southern coastline of the Iberian Peninsula, the climatic suitability is expected to decrease over time. The decrease was projected to be stronger for the scenario RCP8.5.

Temporal shifts regarding the climatic suitability for *Ae. albopictus* could be detected across Europe. The southern parts of Great Britain, Ireland and Sweden were projected to show an increasing climatic suitability over time. The increase was detected to be stronger for the scenario RCP8.5 compared to the scenario RCP4.5. So, for the south-eastern parts of Great Britain, a medium to quite high climatic suitability (values between 0.4 and 0.5) was projected for the 2070s of the scenario RCP8.5. In Germany, areas of increased climatic suitability will first be found in the north-eastern as well as north-western parts and along the Rhine rift valley. These areas of increased climatic suitability are expected to increase over time and the trend is expected to be stronger for the scenario RCP8.5 than for the scenario RCP4.5. In the 2070s (RCP8.5), most parts of Germany were projected to be characterized by a medium to high climatic suitability for *Ae. albopictus* (values between 0.4 and 0.8).

Patterns of high climatic suitability for *Ae. albopictus* were projected for some parts of Europe. A high climatic suitability up to 0.8 was detected for parts of Hungary, Croatia, Serbia and the Danube valley along the border of Bulgaria and Romania over all time periods and scenarios, except for the 2070s in the scenario RCP8.5. For this time period and scenario, the climatic suitability will drop again to values between 0.3 and 0.4. South-east and central France as well as Italy including the Italian islands were projected to be mainly characterized by a quite high climatic suitability (values between 0.4 and 0.8) for *Ae. albopictus*. Here, the 2070s of the scenario RCP8.5 is also expected to be an exception with a decreased climatic suitability.

3.4 Projected climatic suitability for dengue transmission

The climatic suitability for dengue transmission was modeled based on the two IPCC5 scenarios RCP4.5 and RCP8.5 for the three time periods 2021–2040 (2030s), 2041–2060 (2050s), and 2061–2080 (2070s) (Fig. 8). Climatic suitability was expressed as the combination (multiplication) of the climatic suitability for the mosquito and the inverse EIP. In large parts of Europe, the mean climatic suitability for dengue transmission is expected to increase. Furthermore, areas of a certain climatic suitability for transmission was projected to shift northwards. Within each scenario and

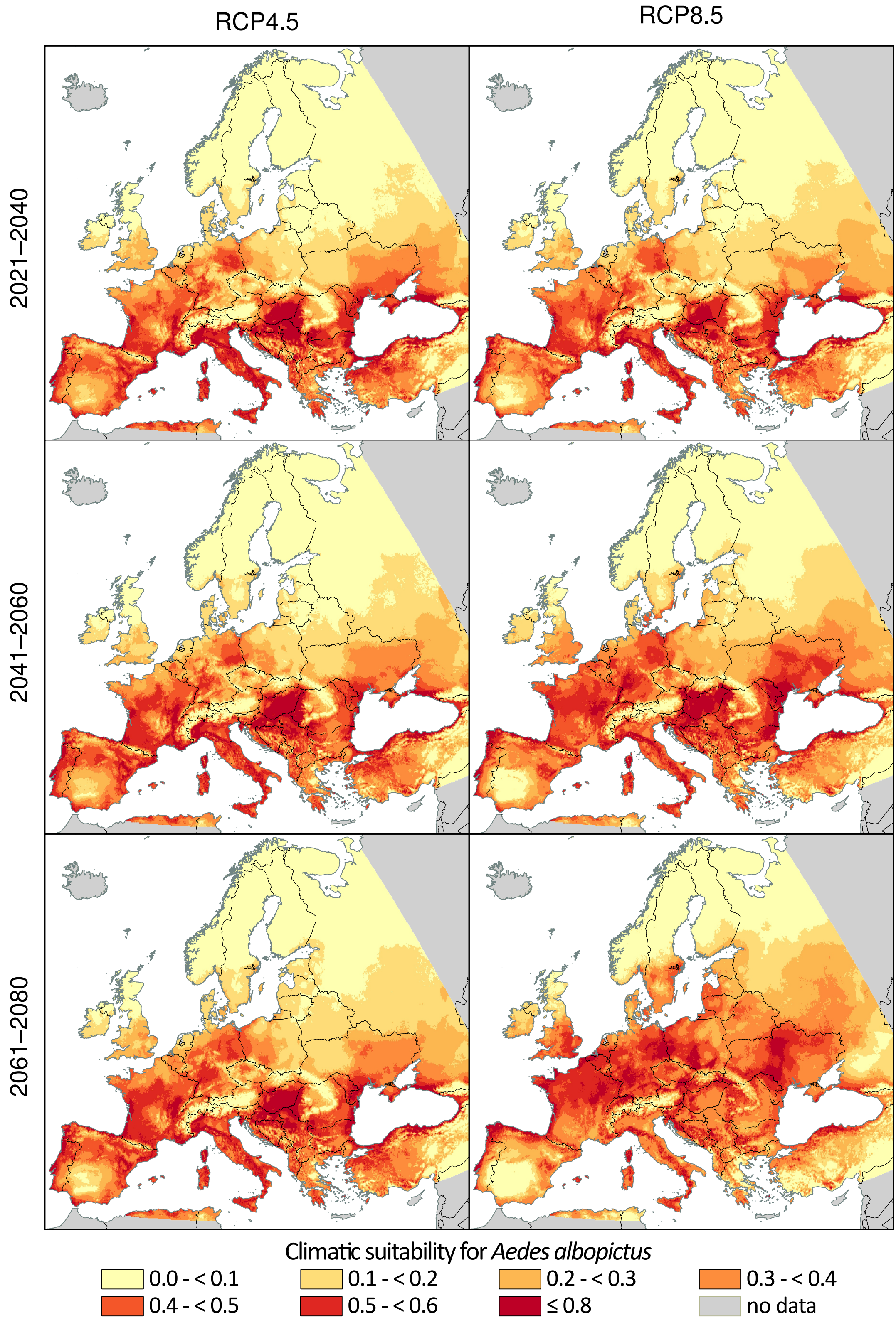

Fig. 7: *Modeled climatic suitability for Ae. albopictus across Europe during the 21st century based on two divergent IPCC5 scenarios (RCP4.5 and RCP8.5). The climatic suitability for Ae. albopictus ranged from 0 (lowest) to 1 (highest). The classes were built in probability steps of 0.1. Values from 0.6 to 0.8 were considered as high climatic suitability and were summarized into one class. No values above 0.8 were yielded.*

time step, the modeled climatic suitability for dengue transmission was generally higher in the south than in the north of Europe.

Some parts of Europe are expected to be characterized by a high climatic suitability for dengue transmission in the future. Areas in Hungary, Croatia, Serbia, south-west of Romania as well as the Danube valley were detected to show a high climatic suitability for dengue transmission (mainly between 0.3 and 0.5). In these areas, the climatic suitability was projected to increase with advancing climate change. An exception was detected for the 2070s in the scenario RCP8.5. Here, the climatic suitability is expected to decrease again. Further areas of high climatic suitability (mainly between 0.3 and 0.5) were projected for the Po valley and the southern tip of Italy, along the coastlines of the Iberian Peninsula, southern France, and Italy, along the Adriatic coastline as well as Black Sea coastline, and on islands in the Mediterranean Sea such as Sicily and Sardinia as well as for the Peloponnese Peninsula. In these areas, the climatic suitability is expected to increase with further advancing climate change with the exception of the 2070s in the scenario RCP8.5. Here, the climatic suitability was detected to decrease again. The climatic suitability for dengue transmission is expected to be very high (exceeding 0.5) in some small areas. Thus, some areas of Europe, such as along the coastline of the Mediterranean Sea, on Mediterranean islands, and along coastline of the Black Sea, were projected to be hotspot areas for dengue transmission.

The climatic suitability for dengue transmission in areas in central and Eastern Europe was projected to increase with advancing climate change. France is expected to turn from an area with very low to low climatic suitability (values between 0.0 and 0.2) to an area with medium climatic suitability (values between 0.1 and 0.4). This trend is found to be more pronounced for the scenario RCP8.5. Here, the south-west coastline was detected to even become an area with a quite high climatic suitability (values between 0.3 and 0.4).

In Germany, the first areas turning from very low to low climatic suitability for dengue transmission (from values between 0.0 and 0.1 to values between 0.1 and 0.2) are expected to be along the upper Rhine rift valley in the 2030s (RCP4.5). Small parts of upland areas in Germany were projected to be characterized by an EIP of more than 60 days in the 2030s (RCP4.5). Thus a climatic suitability for dengue transmission is very unlikely in these areas. The area along the Rhine rift valley with a low climatic suitability (values between 0.1 and 0.2) was detected to expand into areas with formerly very low climatic suitability (values between 0.0 and 0.1). The same is true for expanding areas of low climatic suitability in the north-eastern parts of Germany. This expansion was projected to be stronger for the scenario RCP8.5 compared to the scenario RCP4.5. For the scenario RCP8.5, parts of eastern Germany are expected to turn from very low to low climatic suitability (values between 0.1 and 0.2) already in the 2030s. In the 2070s, large parts of Germany were detected to be characterized by a low climatic suitability (values between 0.1 and 0.2) with medium climatic suitability (values between 0.2 and 0.3) along the upper Rhine valley.

Large parts of Poland and the southern part of Great Britain were projected to be characterized by a low climatic suitability (values between 0.1 and 0.2) by the 2070s (RCP8.5). In the earlier time periods and all time periods of the scenario RCP4.5, the climatic suitability is expected to be mainly very low (values between 0.0 and 0.1) and just in small parts in Poland between 0.1 and 0.2. Regarding areas in the Ukraine and the scenario RCP4.5, little changes were projected compared to the scenario RCP8.5. Here, the areas of medium climatic suitability (values between

0.2 and 0.4) were detected to increase. About half of the Ukraine is expected to turn from very low and low into medium climatic suitability in the 2070s. Areas along the coastline of the Black Sea in the Ukraine were projected to show higher climatic suitability compared to most of the rest of the Ukraine. Along the coastline, the highest climatic suitability was detected during the 2050s before decreasing in the 2070s (RCP8.5).

Parts of the Iberian Peninsula and mountainous regions are expected to show a low climatic suitability for dengue transmission. The valleys of the rivers Guadiana and Gualdalquivir, the Andalusian Plain and parts of the central Iberian Peninsula were detected to show decreasing climatic suitability for dengue transmission over time. This decrease was projected to be stronger for the scenario RCP8.5 than for the scenario RCP4.5. So, in the time period 2070s (RCP8.5), large parts of the Iberian Peninsula are expected to be characterized by a very low climatic suitability (values between 0.0 and 0.1). Higher elevated areas such as the Cantabrian Mountains, Massif Central, Pyrenees, Dinaric Alps, Carpathian Mountains, Caucasus Mountains and Alps were detected to show a lower climatic suitability for dengue transmission compared to the surrounding areas for all observed time periods and both scenarios. These areas of lower climatic suitability compared to surrounding areas were projected to decrease in their extent. This was more pronounced for the scenario RCP8.5 than for RCP4.5.

An EIP of more than 60 days and thus actually no climatic suitability is expected to occur in several European areas, but the extent of many of these areas was detected to decrease in the future. The Pyrenees, the Alps, small parts of the Carpathian Mountains, and the Caucasus Mountains were projected to be characterized by an EIP over 60 days for all three time periods of the scenario RCP4.5 and the time period 2030s of the scenario RCP8.5. Hence, the EIP is expected to exceed the life expectancy of the mosquito and thus nearly no dengue transmission will be possible in these areas. These areas were detected to decrease in the more distant future. The areas of an EIP over 60 days in the Pyrenees are expected to perish considering the scenario RCP8.5 after the 2030s. The Alps and the Caucasus Mountains were projected to show an EIP over 60 days in the 2050s and 2070s of the scenario RCP8.5, too. The extent of these areas was detected to strongly decrease only in the scenario RCP8.5 in the more distant future. A further area with an EIP length over 60 days will be Iceland, where no change in extent was projected. The change in extent was the most expressed in northern parts of Great Britain, and the north-western part of Scandinavia, especially in the Scandinavian Highlands. The decrease was detected to be stronger for the scenario RCP8.5 compared to RCP4.5.

Summing up, areas of lower climatic suitability for dengue transmission in the future were detected in mountainous regions, northern parts of Europe, and the south-western part of the Iberian Peninsula. In contrast, areas with high and increasing climatic suitability with advancing climate change were projected for parts of south-eastern Europe such as Hungary, Croatia, Serbia as well as the Danube valley, and for large parts of Italy, on Mediterranean islands as well as for most parts of the coastline of the Mediterranean and Black Sea.

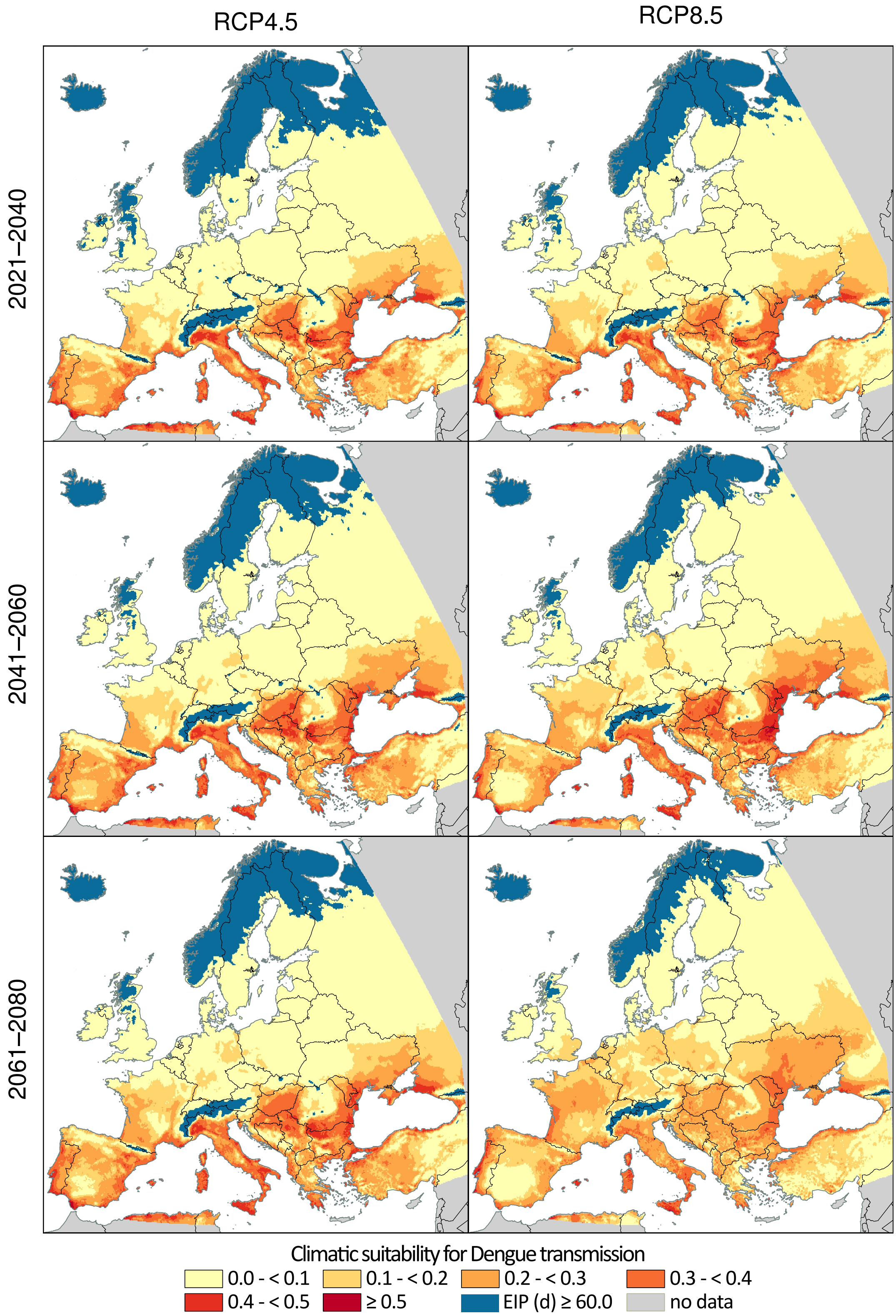

Fig. 8: *Modeled climatic suitability for dengue transmission across Europe during the 21st century based on two divergent IPCC5 scenarios (RCP4.5 and RCP8.5). The classes were built in probability steps of 0.1 up to 0.5. Values above 0.5 were considered as high risk for transmission and were summarized in one class. No values above 0.7 were yielded. EIP values (days) higher than 60 were marked in blue, as the EIP then would exceed the life expectancy of the mosquito Ae. albopictus.*

4 Discussion

The aim of this study was to review the knowledge on the temperature dependence of the EIP of dengue and to develop a general relationship. This should be used to identify future risk areas for dengue transmission across Europe. The results contribute to the discussion, where high risk for dengue transmission will be focused in Europe. Knowledge of such hotspots of dengue transmission is necessary for controlling autochthonous dengue cases and for preventing dengue outbreaks in Europe. First, it was shown that the relationship between temperature and EIP of dengue could best be modeled applying the non-linear unimodal function according to Briere *et al.* (1999). It was projected that the EIP duration is expected to become shorter and the climatic suitability for *Ae. albopictus* to become higher across Europe with advancing climate change. Consequently, the climatic suitability for dengue transmission was detected to become higher.

4.1 Optimizing the model relationship between EIP of dengue and temperature

Regarding the risk for dengue transmission in the temperate zone, knowledge on the EIP at low temperatures is scarce. An EIP at temperatures below 20 °C was only found in two studies which were conducted by McLean *et al.* (1974, 1975). Thus, it was necessary to improve the model fit for the temperature dependence of the EIP. Based on the newly modeled relationship between EIP and temperature, the climatic suitability for dengue transmission in Europe was spatially projected for the 21st century referring to two divergent IPCC5 scenarios (RCP4.5 and RCP8.5).

In order to improve the understanding of the temperature dependence of the EIP of dengue, the current study applied the non-linear left-skewed unimodal function. This approach enabled to take temperature constraints of biological processes at low and high temperatures into account. Temperature constraints of biological processes were previously considered in studies on other vector-borne diseases such as malaria (Briere *et al.* 1999; Paaijmans *et al.* 2009; Mordecai *et al.* 2013; Johnson *et al.* 2015). By applying this non-linear thermodynamic function now to dengue, a temperature impact of low and high temperatures on the physiological processes and traits of the vector mosquito *Ae. albopictus* could be considered (Huey & Stevenson 1979; Angilletta 2009; Dell *et al.* 2011; Amarasekare & Savage 2012). This was important as DENV must replicate within the mosquito, before the virus can be transmitted by the vector to a host (Black *et al.* 2002; Smith *et al.* 2005). Thus, the left-skewed unimodal function represents a model optimization compared to approaches such as linear models by Tjaden *et al.* (2013) or survival analysis by Chan & Johansson (2012).

The main vector mosquito for dengue transmission in Europe is *Ae. albopictus* that is established in southern European countries such as Spain, southern France, Italy, Croatia, and Albania (Adhami & Reiter 1998; Dalla Pozza & Majori 1992; Schaffner *et al.* 2001; Aranda *et al.* 2006; Klobučar *et al.* 2006). Thus, the EIP of dengue regarding the vector *Ae. albopictus* is crucial for modeling the risk for dengue transmission in Europe. Only two recent experimental studies on the EIP of dengue in *Ae. albopictus* could be found (Vega-Rua *et al.* 2013; Xiao *et al.* 2014). However, these experimentally measured EIPs (five out of 38 observations) matched very well the modeled EIP curve of the whole data set. Hence, the modeled temperature dependence of the EIP represented a good indication for the EIP of dengue regarding *Ae. albopictus*.

In almost all published experimental studies, different *Aedes* mosquito provenances were used. In some cases, wild mosquitoes were captured and these or their offspring were used for the experiments (Blanc & Caminopetros 1930; Watts *et al.* 1987; Vega-Rua *et al.* 2013). Partly, the mosquito populations were raised in the lab for more than 30 years (Rohani *et al.* 2009; Xiao *et al.* 2014). For a more precise determination of the EIP under natural conditions only relying on mosquitoes captured in the field would be more promising than lab mosquito populations, because lab-raised mosquitoes can be adapted to the lab conditions (Salazar *et al.* 2007; Tjaden *et al.* 2013). According to Lambrechts *et al.* (2010), mosquitoes colonized in the lab for many generation can bias the experimental results. Moreover, varying mosquito provenances in the experimental studies such as mosquitoes from Mexico, Texas, Bangkok or south-east France can also have an impact on the EIP of dengue (Vega-Rua *et al.* 2013). Since only few studies analyzed field-relevant mosquitoes in their experiments yet (Blanc & Caminopetros 1930; Watts *et al.* 1987; Vega-Rua *et al.* 2013), the source of the mosquitoes could not be further taken into account in the analysis. But the possible bias of geographic origin and intra-specific diversity should not be ignored in future experiments.

Furthermore, almost all studies used a different amount of virus load for the experimental infection of mosquitoes. Moreover, different units for the virus dose were used. This hampers comparability between studies. Only nine for this study relevant experimental studies with all in all 38 observations on the EIP of dengue could be found. Here, the differing amount of viruses and the different designation of the units could not be further considered in the analysis due to the small number of observations. Again, this should be taken into account in the next generation of experiments.

A high initially virus dose can result in a shorter EIP compared to a low virus dose. In reality, only low amounts of viruses are taken up with the insects' blood meal. Additionally, the different methods testing a potential transmission could lead to different resulting EIP values. Measuring the virus uptake in the salivary glands of the mosquito is likely to yield a shorter EIP compared to testing the actual transmission (injection) to a host, because the salivary gland escape barrier is ignored (Black *et al.* 2002; Smith *et al.* 2005). Both, high virus dose and testing only the virus uptake by the salivary glands of the mosquitoes for a potential transmission will lead to conclusions on a shorter mean EIP compared to real transmission situations. Consequently, the risk for dengue transmission based on the EIP will then be overestimated. Since dengue endangers public health, an overestimation of the risk for dengue transmission in Europe is more acceptable than an underestimation of the threat (Tjaden *et al.* 2013).

The temperature dependence of the EIP of dengue was experimentally tested by several authors. After a critical review of these experimental studies, the temperature dependence of the EIP was scrutinized with a novel approach. The applied thermodynamic function was found to optimize the model relationship between EIP of dengue and temperature compared to previous studies (Chan & Johansson 2012; Tjaden *et al.* 2013). This was the basis for the next step, to model EIP durations across Europe under different climate change scenarios.

4.2 Spatial and temporal trends of the EIP of dengue across Europe

The EIP of dengue across Europe was modeled based on the two IPCC5 scenarios RCP4.5 and RCP8.5 for the three time periods 2021–2040, 2041–2060, and 2061–2080. Here, the temporal and spatial development of the EIP of dengue was analyzed across Europe. It could be expected that the EIP will be fulfilled in fewer days with increasing temperature. According to the results, it is likely that the EIP will generally become shorter across Europe. Areas with a short EIP are expected to expand with advancing climate change. Furthermore, north-ward shift of areas with a short EIP was projected. Large areas of European countries, such as Germany or Great Britain, are expected to show a decreased duration of the EIP from long EIP (21–60) to medium EIP (11–21 days). An EIP of 11–21 days can make a difference regarding the transmission potential, because the acquisition of the virus by a vector can be fast enough for a potential transmission to a host. Whereas an EIP of 21–60 days would likely have been too long for a potential transmission of dengue considering the life cycle of the vector. The Iberian Peninsula was projected to show the shortest EIP across Europe and these area was projected to considerably expand in the future. Generally, a short and decreasing EIP could be detected in major river valleys such as the river valleys in Spain, the Danube valley along the border of Bulgaria and Romania as well as the Po valley and in areas along the Mediterranean and Black Sea coastline.

For mountainous and upland regions, such as the Alps, northern Great Britain, and Scandinavia, an EIP of more than 60 days was projected. In areas with an EIP over 60 days, a transmission of dengue is extremely unlikely, as the life expectancy of the vector mosquito will be exceeded (Brady *et al.* 2013). These inappropriate areas are expected to diminish with advancing climate change. Thus, the areas where dengue transmission can categorically be excluded are also expected to be diminished. Regarding the spatial extent of these areas, Scandinavia and Great Britain were detected to show a stronger shrink than the Alps. The Alps are characterized by a higher elevation and by a more dynamic relief. Furthermore, the Alps are distinguished by a longer distance to larger water surfaces compared to northern Great Britain and the Scandinavian Highlands. Thus, a temperature increase and consequently a shortened EIP duration in northern Great Britain and Scandinavia can be stronger due to a shallower terrain and the closeness to the Atlantic Sea, Norwegian Sea, and Baltic Sea. The mountainous region of the Pyrenees was projected to show a shorter EIP than 60 days from the time step 2041–2060 of the higher emission scenario RCP8.5. Here, the vicinity of the Atlantic Sea and Mediterranean Sea might also have an influence on the stronger temperature increase compared to the Alps.

Similar patterns of a decreasing EIP across Europe (especially in the south of Europe) and a northward shift of areas with a shortened EIP were also detected by Thomas *et al.* (2011). In comparison with Thomas *et al.* (2011), the data in the current study are based on a larger number of experimental studies on the EIP of dengue and an optimized statistical modeling of the temperature dependence of the EIP. Thus, a more adequate analysis of the EIP across Europe was possible. Further, the current study provided a more detailed overview of the EIP and the risk for dengue transmission in Europe. As a matter of fact, the applied model for the EIP was based on the new IPCC5 emission scenarios (IPCC 2013) that were not available for Thomas *et al.* (2011). A further progress of the current study is the combination of EIP of dengue with the climatic suitability for *Ae. albopictus* to project the potential for dengue transmission.

In conclusion, the hypothesis that the duration of the EIP will decrease across Europe can be accepted. An increasing extent and north-ward shift of areas with short EIP were projected. For an analysis of the risk for dengue transmission, the modeled EIP across Europe is not enough, as the presence of the vector mosquito *Ae. albopictus* plays also an important role for the transmission process.

4.3 Combined assessment of the EIP of dengue and the climatic suitability for *Aedes albopictus*

A combined assessment of the EIP of dengue and the climatic suitability for *Ae. albopictus* was applied to model the climatic suitability for dengue transmission across Europe. Here, it was analyzed whether the climatic suitability for dengue transmission will be enhanced across Europe due to an increasing climatic suitability for the vector and a shortened EIP with advancing climate change. It was further tested, whether areas of high climatic suitability for dengue transmission will expand and whether these areas can be expected further north.

Modeling the climatic suitability of *Ae. albopictus*, the temperature parameters annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, mean temperature of the driest, warmest and the coldest quarter were considered. Minimum temperatures could be a restricting factor regarding the overwintering success of the mosquitoes (Medlock *et al.* 2006; Straetemans 2008; Thomas *et al.* 2012). In the model for the climatic suitability of *Ae. albopictus* no precipitation parameters were taken into account. However, maximum temperatures during the driest or warmest quarter or month not only reflects the highest temperatures during a year, but can also reflect water availability for the mosquitoes. High temperatures during the driest and warmest quarter can lead to higher evaporation and thus could lead to a lower water availability. According to the results, the climatic suitability for *Ae. albopictus* is expected to become generally higher across Europe, especially in southern and central Europe. Exceptions are mountainous regions such as the Alps as well as the Carpathians and large parts of Spain as well as Greece. Mountainous regions are characterized by too cold temperatures for *Ae. albopictus* due to higher elevation. The extent of the mountainous regions with low climatic suitability was detected to diminish in the future. In contrast, the climatic suitability for *Ae. albopictus* in large parts of Spain and Greece was projected decrease with advancing climate change. This trend of a decreasing climatic suitability is induced by decreasing precipitation and increasing evaporation in the future due to climate change.

During summer, water availability and precipitation can be a limitation for the breeding success of *Ae. albopictus* (Mitchell 1995; Straetemans 2008). According to Mitchell (1995) and Straetemans (2008), a precipitation of at least 300 mm to 500 mm is required for successful colonization. The necessity of a certain amount of precipitation is questionable, as *Ae. albopictus* is able to breed in small artificial containers filled with some water such as catch basins, tanks, and tires (Hawley 1988; Mitchell 1995). These types of breeding sites are also available in dry areas, such as dry urban areas or gardens due to watering of these areas. The eggs of *Ae. albopictus* show also a high desiccation tolerance (Scholte & Schaffner 2007). Thus, the low climatic suitability in areas such as large parts of central Spain has to be taken with caution. The modeled climatic suitability can hence underestimate the presence of *Ae. albopictus* in these areas.

Combining the modeled EIP across Europe and the climatic suitability for *Ae. albopictus*, an increase in climatic suitability for dengue transmission across Europe could be detected. Further, areas with high climatic suitability are expected to expand and a north-ward shift of these areas was projected. The climatic suitability was detected to be high especially in areas along the Black Sea coastline, the Mediterranean coastline, on Mediterranean islands, along the Atlantic coastline in Portugal as well as south-west France, and in large parts of Italy with advancing climate change starting already in the time step 2021–2040. Thus, these areas could be designated as hotspots for dengue transmission in Europe already in a few years.

In several European countries, such as Germany, Poland, and southern Great Britain, the climatic suitability was projected to increase from very low to low and medium climatic suitability. Hence, more and more areas are expected to be affected by an increased climatic suitability for dengue transmission. Although the climatic suitability in these areas is expected to still remain to be relatively low, it is important to observe the development and potential dengue transmission especially in areas of favorable microclimatic conditions. In Germany, areas such as the Rhine rift valley were detected to show an enhanced climatic suitability for dengue transmission compared to the surrounding area and thus can be an entrance point for dengue into Germany in the future. Mountainous regions, also in southern Europe, were projected to show a very low climatic suitability for dengue transmission, as the EIP will be quite long and the climatic suitability for the vector will be low. Further exceptions of a low climatic suitability in southern Europe were detected for parts of Greece, and south-western as well as central parts of the Iberian Peninsula. In these areas, the climatic suitability is expected to even decrease with advancing climatic change. Here, the EIP was projected to be the shortest across Europe, but the climatic suitability for the vector *Ae. albopictus* is expected to remain very low. Summarizing, the hypothesis of increasing climatic suitability for dengue transmission in Europe, of expansion of areas with high climatic suitability as well as of a northward shift of suitability can be accepted with the exception of the Iberian Peninsula. Nevertheless, the future climatic suitability for dengue transmission on the Iberian Peninsula might be underestimated.

Liu-Helmersson *et al.* (2014) modeled the global dengue epidemic potential including temperate regions. Bouzid *et al.* (2014) analyzed the risk for dengue fever in Europe. Similar to the current study, both studies were interested in the potential for dengue transmission in Europe, but had a different approach with more parameters included in the model. Liu-Helmersson *et al.* (2014) used the parameters mortality rate, biting rate, infection probability of the mosquitoes by humans, dengue transmission probability to humans by the vector mosquitoes, and the extrinsic incubation period. However, all of the vector parameters were based on only few studies of *Ae. aegypti*, whereas *Ae. albopictus* and not *Ae. aegypti* is the vector for dengue on the European mainland. Due to the low amount of studies, the data basis for estimating each parameter was small. Furthermore, the authors recognized that the applied parameter estimation and available data basis was inconsistent due to non-standardized data sampling. Here, the future projections were based on the IPCC5 scenario RCP8.5 (highest radiative forcing) for the time period 2070–2099. Hence, no development of the vector competence across different time steps could be detected. In addition, the presence of the vector *Ae. aegypti*, which is required for dengue transmission, was not considered. According Liu-Helmersson *et al.* (2014), the potential for dengue transmission was projected to increase in most parts of Europe including parts of Scandinavia such as Sweden and Finland. This is in contrast to the results of the current study. Combining the climatic suitability of *Ae. albopictus* with the modeled EIP across Europe, the climatic suitability for dengue transmission is expected to be very low in Scandinavia in the time period 2061–2080 (RCP8.5). A very low climatic suitability for dengue transmission was projected for whole Scandinavia, although the climatic suitability for the vector *Ae. albopictus* was detected to increase in southern Sweden with advancing climate change. Thus, the potential for dengue transmission according to Liu-Helmersson *et al.* (2014) might be overestimated because the temperature dependence of the EIP probably contributed only marginally to their model. They focused probably too much on the characteristics of the vector.

For the risk assessment of dengue fever in Europe, Bouzid *et al.* (2014) considered parameters such as dengue incidences in Mexico and their underlying climatic parameters, the yearly gross domestic product, and the population density. Bouzid *et al.* (2014) modeled the risk for dengue transmission based on the relationship between dengue incidences and environmental parameters observed in Mexico for the three time steps 2011–2040, 2041–2070, and 2071–2100 based on the IPCC emission scenario A1B. The modeled number of dengue cases and the modeled dengue fever incidence rate were detected to be high especially in densely populated areas in southern Europe such as the areas around Sevilla, Valencia, Barcelona, Marseille, Milano, and Roma. The eastern coastline of Italy was projected to show an increased number of dengue cases. For the rest of the countries such as France and Spain, for Greece, and the coastline of the Black Sea, the number of dengue cases was detected to be very low. Only the more densely populated areas showed a slightly increased risk. These results are in contrast to the results from the current study, where nearly whole Italy and the coastline of the Black Sea is expected to be characterized by a high climatic suitability for dengue transmission. Further, in Greece, France and Spain, larger parts compared to the study by Bouzid *et al.* (2014) were projected to show an enhanced climatic suitability for dengue transmission. Since very densely populated cities were detected to show an increased number of dengue cases and an increased dengue fever incidence rate according to Bouzid *et al.* (2014), the impact of the population data considered in the model might be to high compared to the climatic parameters required for dengue transmission. Since the climatic parameters were calibrated to dengue incidences in Mexico, the transmission potential can be underestimated. No information on temperature requirements in the temperate zone was considered. Evidence suggests, that dengue can also be transmitted at lower temperatures than those prevailing in Mexico.

Regarding a potential transmission of dengue in Europe, a vertical transmission of the virus from adult mosquito to offspring and locally occurring adaptation between vector and virus could increase the risk of a larger dengue outbreak. Both vector mosquitoes *Ae. aegypti* and *Ae. albopictus* are able to transmit DENV vertical from the adult mosquito to its offspring (Joshi *et al.* 2002; Martins *et al.* 2012; Buckner *et al.* 2013; Espinosa *et al.* 2014). A vertical transmission of DENV can increase the persistence of the virus in an area. This could enhance the risk of a dengue outbreak due to vectors that inherited the infection (Buckner *et al.* 2013). Hence, DENV could be passed from one mosquito generation to another one. So, the virus could persist in an area where no humans are infected by DENV during an interepidemic period (Joshi *et al.* 2002; Espinosa *et al.* 2014).

A possible adaptation of the virus to one vector can enhance the potential of virus transmission in Europe. Vega-Rua *et al.* (2014) found that the transmission efficiency of *Ae. aegypti* and *Ae. albopictus* differed depending on the transmitted virus strain and genotype of CHIKV. *Ae. albopictus* showed a higher efficiency transmitting the mutant strain CHIKV from East-Central-South Africa than *Ae. aegypti*. In contrast, *Ae. aegypti* transmitted the original CHIKV strain better. On islands in the Indian Ocean, CHIKV showed an adaptive mutation to match transmission requirements of *Ae. albopictus* and to improve replication in a new vector (De Lamballerie *et al.* 2008). Tsetsarkin *et al.* (2007) pointed out that mutations of proteins of CHIKV could lead to a faster dissemination, higher infectivity and thus to an increased transmission efficiency of one vector mosquito (e.g. *Ae. aegypti*) compared to an other vector mosquito (e.g. *Ae. albopictus*). This could result in an enhanced vectorial capacity. Hence, it could result in larger CHIK outbreaks in regions such as Europe where the typical vector *Ae. aegypti* of CHIKV is absent. According to Lambrechts *et al.* (2009), a local adaptation of DENV to the vector *Ae. aegypti* is possible and thus such kind of selection can play an important role regarding the transmission rate of dengue. Anderson & Rico-Hesse (2006) analyzed different genotypes of DENV. DENV could be earlier detected in the salivary glands of *Ae. aegypti* which were infected by the Southeast Asian genotype compared to the American genotype. Hence, the vectorial capacity was enhanced for the mosquitoes infected by the Southeast Asian genotype. Vega-Rua *et al.* (2013) compared the transmission efficiency of DENV by the tropical vector *Ae. aegypti* and the vector *Ae. albopictus* from France. Compared to *Ae. aegypti* from Martinique, a higher percentage of the European vector *Ae. albopictus* was able to transmit DENV after the same incubation period.

In summary, several studies indicated that an adaptation of DENV to local mosquitoes is possible and can lead to larger dengue outbreaks in Europe. Since detailed information is yet missing, this could not be considered in the applied model and in no other existing model on dengue transmission, yet. Thus, the risk of dengue outbreaks can be underestimated due to locally occurring adaptation of virus and vector, which could not be taken into account.

4.4 Discussion of methodology

The presented study managed to improve the picture on the EIP of dengue, climatic suitability for *Ae. albopictus*, and the climatic suitability for dengue transmission in Europe. For models of the current climate and the future climate of the study area, the climatic data were derived from WorldClim (`http://worldclim.org`, Hijmans *et al.* 2005). Here, it has to be mentioned that weather stations are not evenly distributed across Europe, especially rural areas and natural landscapes are underrepresented in general. The density also depends on the economic state of countries. The climate data had to be interpolated due to only few weather stations in parts of northern and eastern Europe (Hijmans *et al.* 2005). Thus, the projected climate for Europe based on worldclim data can deviate from the climate in reality. Further, the daily resolved climate data for the future projections of dengue transmission in Europe were provided by EURO-CORDEX and had a spatial resolution of 0.11 deg. These climate data on regional spatial scales with a relatively high resolution enable a small-scaled analysis compared to global models. Nevertheless, small local differences, such as in the Alps between valleys and mountain summits, cannot be detected.

These differences can have an effect on projections of the climatic suitability for *Ae. albopictus* like in Switzerland (Neteler *et al.* 2013). Neteler *et al.* (2013) pointed out that *Ae. albopictus* was already found in southern parts of Switzerland in 2012, whereas a very low climatic suitability for *Ae. albopictus* in southern Switzerland was projected by the current study. This illustrates the problems of topographic heterogeneity for model projections based on coarse resolution climate models.

In the current study, daily temperatures were used in the model of the EIP across Europe. According to Lambrechts *et al.* (2011) and Carrington *et al.* (2013b), an increased diurnal range is likely to reduce the probability of dengue infection of female vector mosquitoes. In contrast, the potential transmission rate for dengue will be underestimated in the temperate zone, when considering mean temperatures and not the diurnal temperature range (Carrington *et al.* 2013a). In areas with low mean temperatures such as the temperate zone, diurnal temperature fluctuation have a positive influence on the transmission of dengue due to a shortened EIP and lower mortality rates of the vector (Carrington *et al.* 2013a). Further, Liu-Helmersson *et al.* (2014) pointed out that the dengue transmission potential in the temperate zone will be simulated by an increasing diurnal temperature range. In the current study, daily temperatures and not the diurnal temperature range were considered due to data availability. Thus, it has to be taken into account that the risk for dengue transmission might be higher than it is represented by the modeled climatic suitability for dengue transmission in the current study.

Besides the parameters which were considered in the this study, further ecological and biological parameters show an influence on the transmission of dengue. This could be the body size, reproduction rate, mortality rate, flying activity as well as biting rate of the vector mosquitoes, mosquito abundance, an infection probability of the mosquitoes by humans and a dengue transmission probability to humans by the mosquitoes (Lambrechts *et al.* 2011; Crepeau *et al.* 2013; Liu-Helmersson *et al.* 2014; Williams *et al.* 2014). Thus, more parameters should be included when aiming to improve modeling the potential of dengue transmission across Europe. However, considering many parameters in a model has to be taken with care, as the model could suffer from overprediction. Moreover, inconsistency of the data can be high when using several parameters based on different data sources, because each study focuses on aspects of parameters and applies different methods. This problem occurred in the study of Liu-Helmersson *et al.* (2014). The current study avoided over parametrization and inconsistencies by restricting model parameters and by modeling the EIP based on broad literature analysis. It was focused on the EIP, as the influence of EIP on the potential of dengue transmission is high in areas with a suitable climate for *Ae. albopictus* (Focks *et al.* 1995).

Finally, it has to be considered that the study modeled the impact of climate change on the potential of dengue transmission in Europe. Thus, human population data such as population density were not taken into account. With an increasing human population density, the vector-host-contact increases. As *Ae. albopictus* is an opportunistic feeder, an increased contact to humans in densely populated areas facilitates dengue outbreaks. Further, DENV as well as the vector *Ae. albopictus* was and will be introduced to Europe via trade and travel. Regarding the autochthonous cases in Europe in the last years, DENV was introduced by travelers coming from dengue endemic areas (La Ruche *et al.* 2010; Schmidt-Chanasit *et al.* 2010; Marchand *et al.* 2013; Wilder-Smith *et al.* 2014). The vector mosquito *Ae. albopictus* was introduced to Europe via trade of goods such as used tires as well as ornamental plants and via travel by planes, ships as well as cars (Knudsen

1995; Scholte & Schaffner 2007; Scholte *et al.* 2008; Straetemans 2008). Thus, international hubs such as large airports, harbors as well as railway stations and highways coming from *Ae. albopictus* and DENV endemic areas are entrance points for the virus and its vector into Europe. Hence, the introduction of the vector and DENV to Europe is likely to proceed further due to globalization and increasing international trade and travel activities (Jelinek 2009; Simmons *et al.* 2012; Semenza *et al.* 2014).

To model the potential of dengue transmission in Europe, the current study focused on the EIP and the climatic suitability for *Ae. albopictus.* Applying this approach, a broad overview of the potential of dengue transmission could be given and the impact of climate change on dengue transmission could be detected. For further studies, it is recommended to combine this modeled potential for dengue transmission with human population data. Thus, the analysis of the impact of climate change could be extended by further research on anthropogenic impact such as human population density or travel and trade trajectories.

4.5 Implications of an increased risk for dengue transmission in Europe

A shortened EIP, increasing climatic suitability for *Ae. albopictus* and increasing climatic suitability for dengue transmission in Europe was projected in the current study. Hotspots for dengue transmission are expected mainly along the coastline of the Mediterranean Sea, on Mediterranean islands, in large parts of Italy and along the coastline of the Black Sea. These regions are popular tourist areas due to relatively high temperatures during summer and the closeness to the sea. Thus, many travelers from all over Europe are staying in these regions during the summer months. An increased travel activity and population density in combination with the season (May–November) for the vector mosquito *Ae. albopictus* can facilitate and enhance the risk and spread of dengue (Schmidt-Chanasit *et al.* 2010).

Vector-borne-disease outbreaks can be handled differently, as it was shown during disease occurrences in France and Italy. The CHIKV outbreak in Italy in 2007 with more than 200 recorded cases showed that larger outbreaks of vector-borne-diseases are possible in Europe (Angelini *et al.* 2007; Rezza *et al.* 2007). In the affected region, measures for controlling the vector *Ae. albopictus* and a surveillance system was set up (Angelini *et al.* 2007; Rezza *et al.* 2007). Insecticides as well as insect growth regulators were applied and breeding sites were eliminated (Angelini *et al.* 2007; Rezza *et al.* 2007). Thus, the CHIK outbreak was limited despite the high number of cases and did not affect adjacent areas. Similar measures can be able to limit dengue outbreaks in Europe.

Autochthonous dengue cases in southern France in 2010 and 2013 could be restricted to two and one cases (La Ruche *et al.* 2010; Marchand *et al.* 2013). Here, a national plan against a further spread of dengue was implemented. Vector control measures such as the use of insecticides as well as repellents, destruction of breeding sites, installation of traps and larvicide treatments were set up. Further, information about dengue for the public and physicians were provided. Health professional have been sensitized regarding diagnoses of dengue cases. An active case finding program in the neighborhood of the autochthonous dengue cases was implemented. Finally, control measures at the harbor and international airport were set up. This shows that politics require knowledge on vector-borne-diseases and a management plan should be implemented which can be applied, when autochthonous dengue cases occur. Control measures regarding the vector *Ae. albopictus* should be set up to restrict further establishment of the vector.

The last cases of CHIK and DENV indicated how important it is to react fast in the case of dengue incidences. In general, control measures regarding the vector *Ae. albopictus* at harbors and airports and a monitoring system are important to slow down the introduction and establishment of *Ae. albopictus* in new areas. Monitoring of the established *Ae. albopictus* is needed for a better application of control measures when autochthonous dengue cases occur (Petrić *et al.* 2014). Once *Ae. albopictus* is introduced and established, it is very hard to control the established populations of *Ae. albopictus*. Further, travelers need to be informed about the risk of dengue in dengue endemic areas and health professional need to be aware of diagnoses of dengue (Marchand *et al.* 2013). Physicians have to be vigilant regarding travelers with fever coming from dengue endemic areas. This is especially important in areas with a high climatic suitability for dengue transmission.

Summing up, the potential for dengue transmission in Europe is increasing, especially in the touristic areas in southern Europe. Hence, dengue is not a tropical disease any more, but can occur in the temperate zone. Thus, information of the public as well as travelers about dengue and an increasing awareness by health professionals regarding dengue diagnoses are important. These measures are important, as a vaccine against dengue is still not developed (World Health Organization 2014).

5 Conclusion

The present study showed that the risk of dengue fever in Europe can be expected to increase with advancing climate change. In large parts of Europe, the climatic suitability for dengue transmission was projected to be low to medium, but also hotspots with a very high climatic suitability were detected up to the end of the century. These hotspots are expected along the Mediterranean coastline, on Mediterranean islands, in large parts of Italy and along the Black Sea coastline. Thus, hitherto unknown autochthonous transmissions of vector-borne diseases, such as DENV, will no longer be restricted to tropical and subtropical regions. The projected increasing risk for dengue transmission in Europe should be taken as a very serious threat to public health.

Hotspots for dengue transmission were detected to be especially in touristic areas, where an increased human population density during summer overlaps with the season of the vector *Ae. albopictus*. Further, travel activity is expected to be increased in these areas. Thus, a transmission of dengue can be facilitated through human population density in addition to the high climatic suitability for dengue transmission in these areas. Hence, management plans including control measurements and surveillance systems should be particularly focused on these hotspot areas. Moreover, these hotspot areas can act as entrance points for the vector *Ae. albopictus* and DENV to other European areas in the case of a dengue outbreak. Thus, monitoring should be set up to recognize outbreaks and to avoid the spread of dengue in Europe.

Dengue is no longer a mere tropical disease, in the near future it can become a threat for Europe. In consequence, further research regarding the potential of dengue transmission is needed. In the current study, the impact of climate change on the transmission of dengue was analyzed. The achieved results can now be extended by combining them with analysis on the anthropogenic impact such as human population density and international connectivity. These parameters can be supporting model predictors, because Europe is relatively densely populated and internationally well connected via large harbors and airports. The possibility of adaptation of DENV to local mosquitoes should also be investigated. Adaptation would increase the risk for dengue transmission and then would facilitate larger dengue outbreaks in Europe. Besides further research, travelers have to be informed about dengue and health professionals need to be aware of diagnoses of dengue, especially in the hotspot areas. In addition, politicians should gain knowledge on vector-borne diseases such as dengue to implement efficient management plans that can be immediately applied when dengue cases occur in Europe.

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Appendix

The spatial patterns of temperature and precipitation and the projected climate in Europe were provided, as these climatic variables influence the EIP and the climatic suitability for *Ae. albopictus*. For the climatic suitability of *Ae. albopictus*, the annual mean temperature, the temperature of the warmest, and the temperature of the coldest month play an important role. The temperature of the warmest month is also important for the EIP, as the EIP becomes shorter with increasing temperature. The climatic parameter annual precipitation was chosen to get an impression of the changing precipitation patterns due to climate change in Europe. Changing precipitation patterns might influence the climatic suitability for *Ae. albopictus* and consequently the risk of dengue fever in Europe.

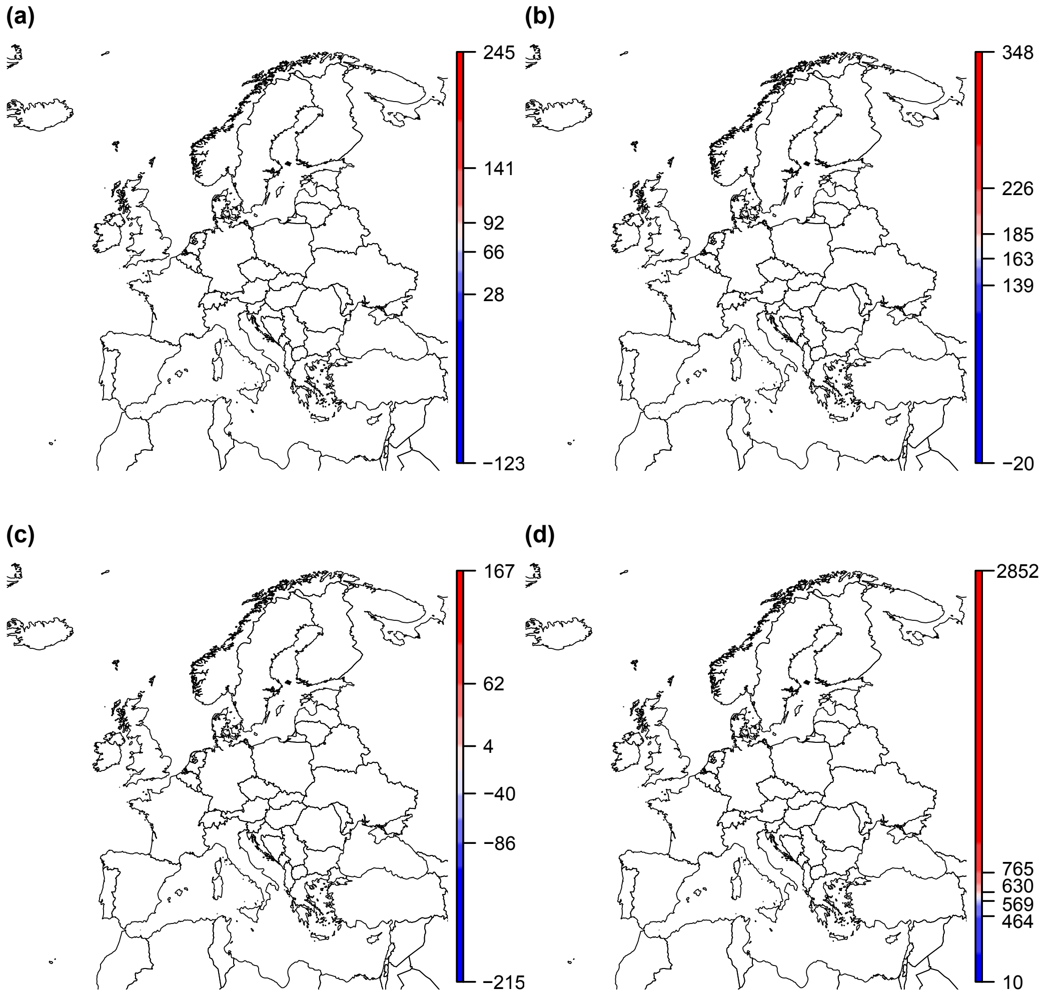

Fig. A1: *Spatial patterns of temperature and precipitation in Europe. The temperature variables (a) annual mean temperature, (b) mean temperature of the warmest month, and (c) mean temperature of the coldest month are given in* d°C *(*1/10°C*), (d) annual precipitation is given in mm. The color classes represent 10th percentiles of the data. The legend labels represent the 20th percentiles. The spatial resolution is 5 arcminutes and the coordinate reference system is WGS84. Data source: Hijmans* et al. *(2005).*

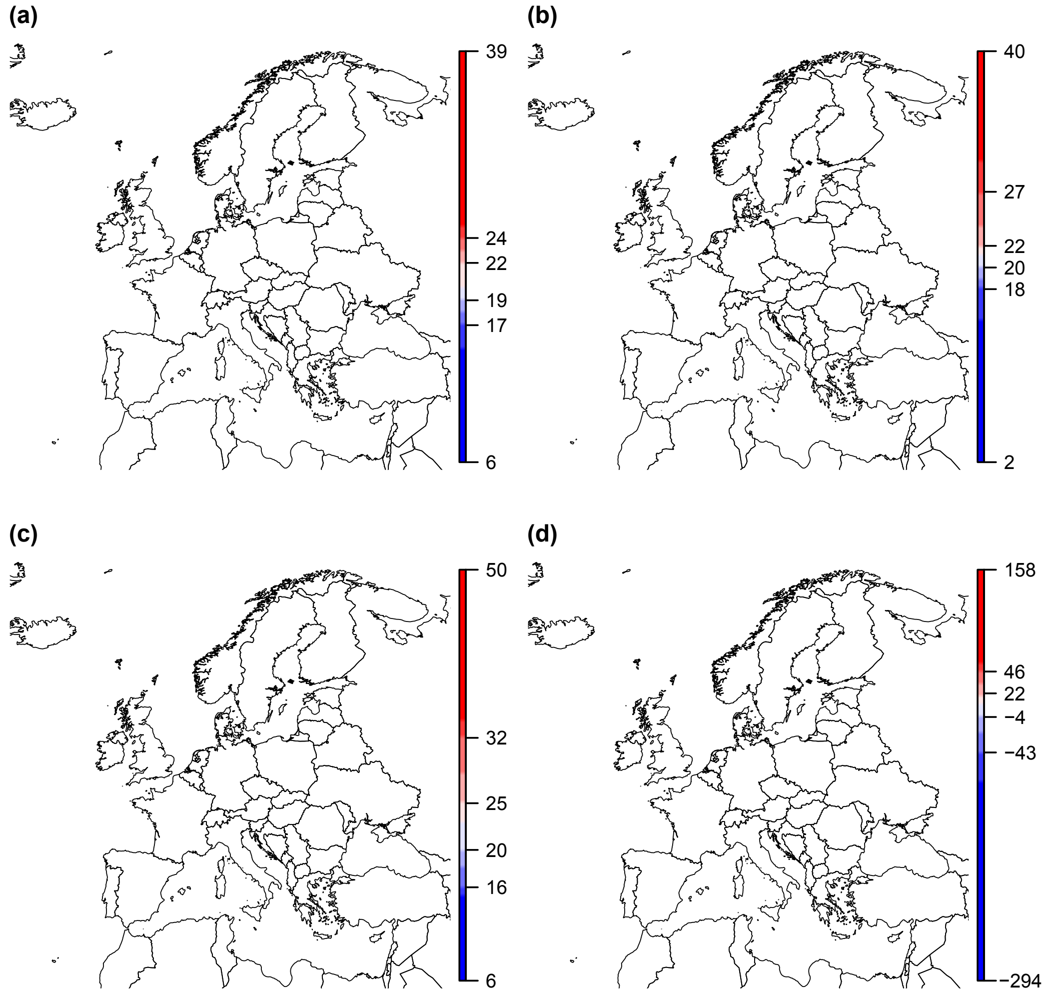

Fig. A2: *Projected climate in Europe for the scenario rcp4.5 and time step 2041 - 2060. The current climatic parameters were subtracted from the projected climatic parameters to visualize the actual change. The temperature variables (a) annual mean temperature, (b) mean temperature of the warmest month, and (c) mean temperature of the coldest month are given in* d°C (1/10°C)*, (d) annual precipitation is given in mm. The color classes represent 10th percentiles of the data. The legend labels represent the 20th percentiles. The spatial resolution is 5 arcminutes and the coordinate reference system is WGS84. Data source: Hijmans* et al. *(2005).*

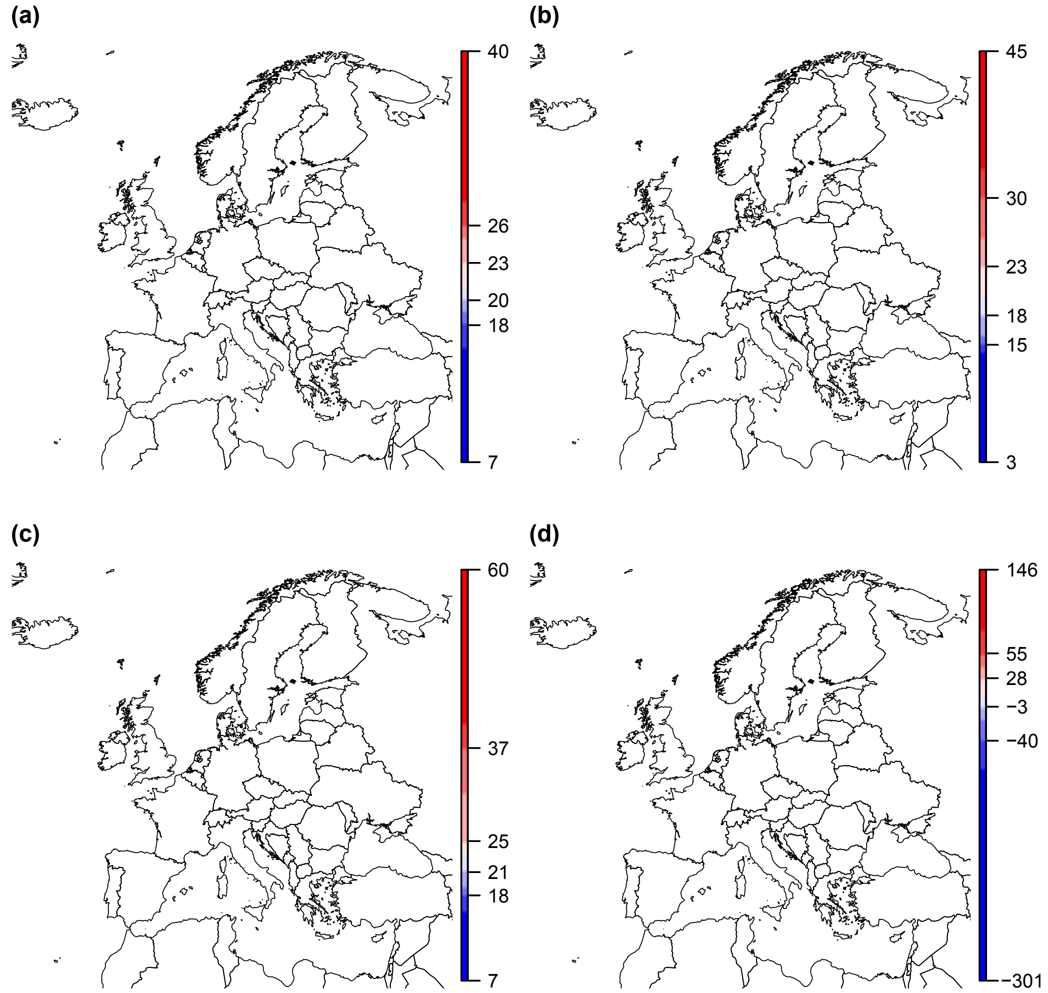

Fig. A3: *Projected climate in Europe for the scenario rcp4.5 and time step 2061 - 2080. The current climatic parameters were subtracted from the projected climatic parameters to visualize the actual change. The temperature variables (a) annual mean temperature, (b) mean temperature of the warmest month, and (c) mean temperature of the coldest month are given in* d°C (1/10°C)*, (d) annual precipitation is given in mm. The color classes represent 10th percentiles of the data. The legend labels represent the 20th percentiles. The spatial resolution is 5 arcminutes and the coordinate reference system is WGS84. Data source: Hijmans* et al. *(2005).*

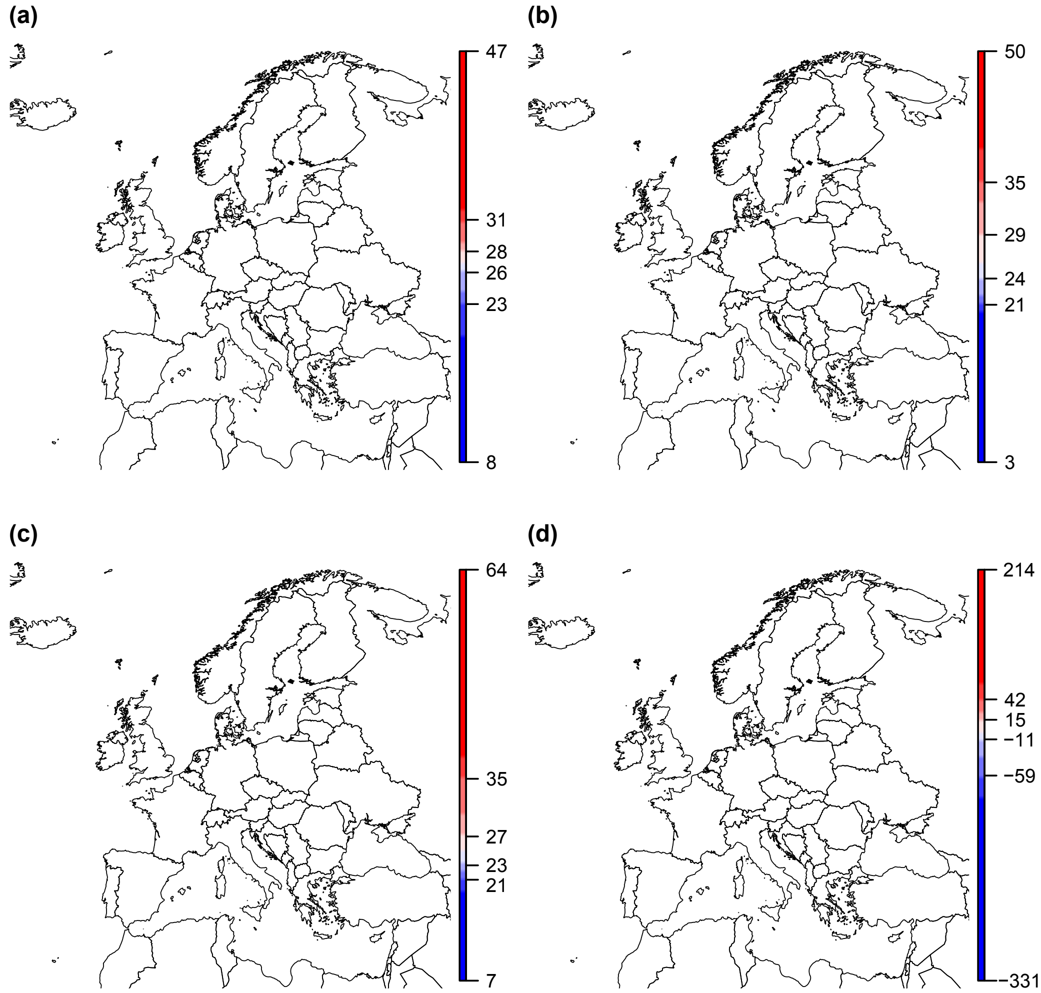

Fig. A4: *Projected climate in Europe for the scenario rcp8.5 and time step 2041 - 2060. The current climatic parameters were subtracted from the projected climatic parameters to visualize the actual change. The temperature variables (a) annual mean temperature, (b) mean temperature of the warmest month, and (c) mean temperature of the coldest month are given in* d°C (1/10°C)*, (d) annual precipitation is given in mm. The color classes represent* 10^{th} *percentiles of the data. The legend labels represent the* 20^{th} *percentiles. The spatial resolution is 5 arcminutes and the coordinate reference system is WGS84. Data source: Hijmans* et al. *(2005).*

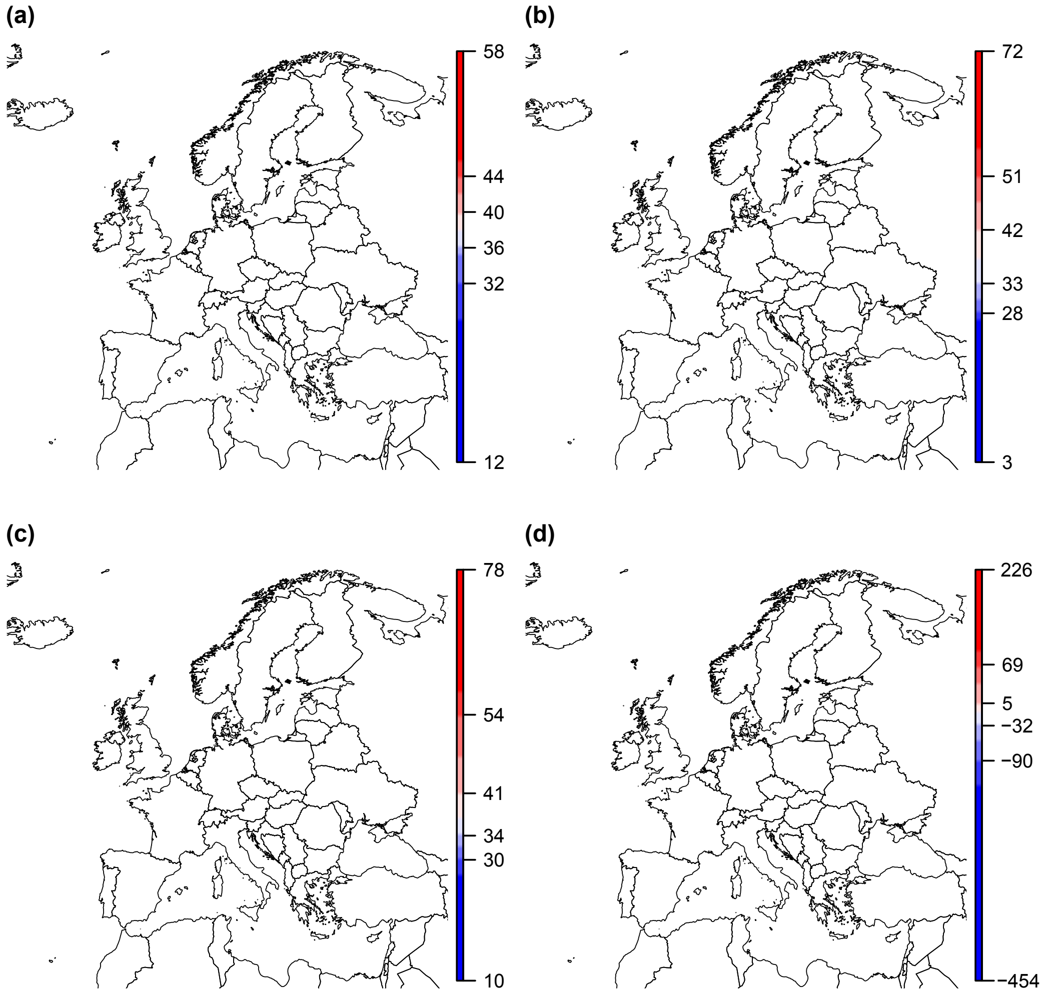

Fig. A5: *Projected climate in Europe for the scenario rcp8.5 and time step 2061 - 2080. The current climatic parameters were subtracted from the projected climatic parameters to visualize the actual change. The temperature variables (a) annual mean temperature, (b) mean temperature of the warmest month, and (c) mean temperature of the coldest month are given in* d°C (1/10°C)*, (d) annual precipitation is given in mm. The color classes represent 10th percentiles of the data. The legend labels represent the 20th percentiles. The spatial resolution is 5 arcminutes and the coordinate reference system is WGS84. Data source: Hijmans* et al. *(2005).*

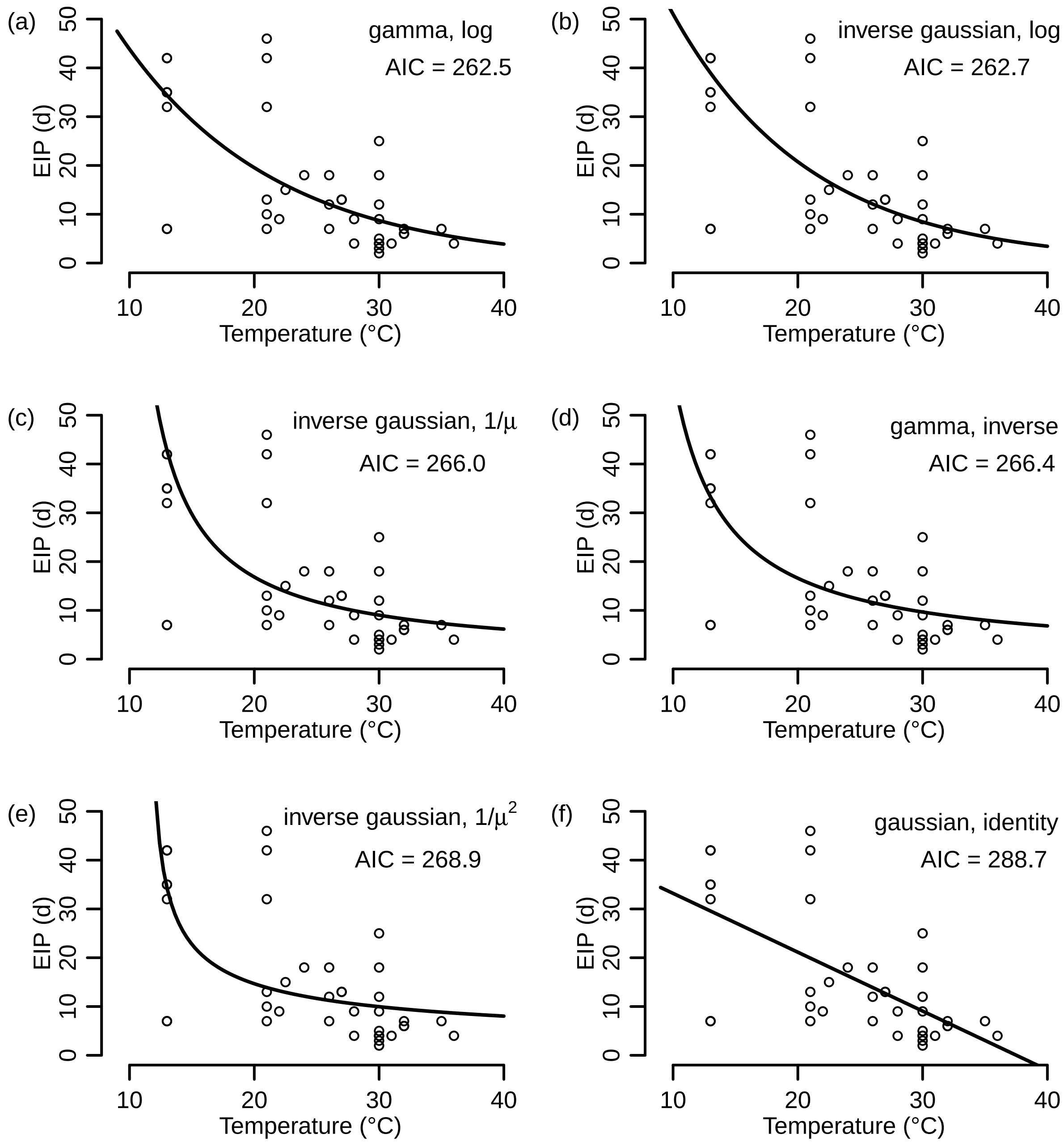

Fig. A6: *Modeled relationship between EIP of dengue and temperature applying general linear models. Each point gives the time interval between infection of the vector mosquito Ae. aegypti or Ae. albopictus and a potential transmission of dengue by the vector (N = 38). The vector mosquitoes were infected by feeding on infected animals or artificial blood meals containing the virus. According to the given labels, different distributions and link functions were tested. To compare the quality of the models assuming different distributions, the Akaike Information Criterion (AIC) was considered. The lower the AIC value is, the higher is the probability assuming the right distribution.*

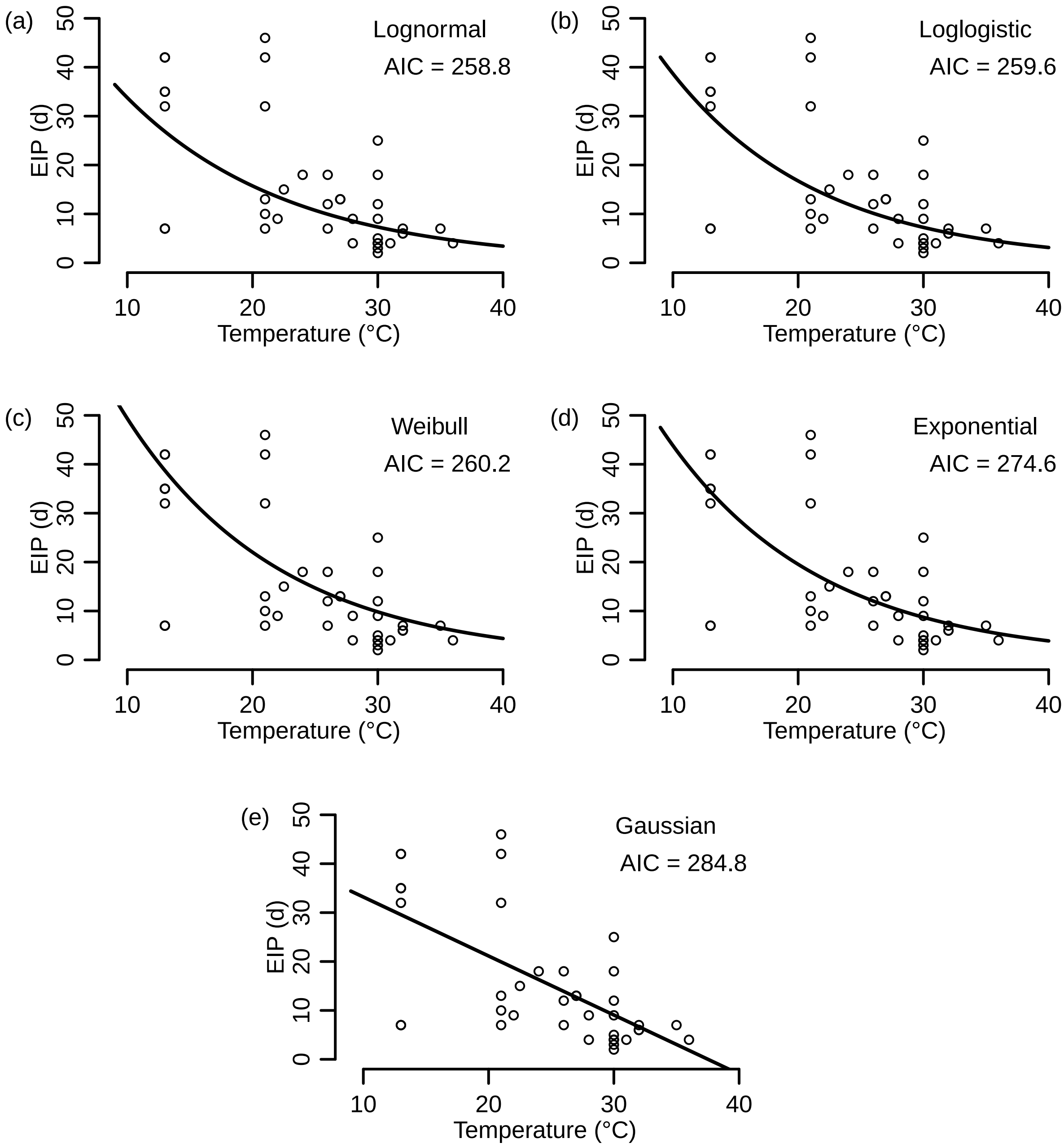

Fig. A7: *Modeled relationship between EIP of dengue and temperature applying survival analysis. Each point gives the time interval between infection of the vector mosquito Ae. aegypti or Ae. albopictus and a potential transmission of dengue by the vector (N = 38). The vector mosquitoes were infected by feeding on infected animals or artificial blood meals containing the virus. According to the given labels, different distributions and link functions were tested. To compare the quality of the models assuming different distributions, the Akaike Information Criterion (AIC) was considered. The lower the AIC value is, the higher is the probability assuming the right distribution.*

Tab. A1: *Reviewed data on the EIP of dengue and temperature. The time interval between infection of the vector mosquito by feeding and a potential transmission of dengue by the vector was analyzed at a given temperature. The reviewed studies are described by their first author and the year of publication. Further, the analyzed vector mosquito species and the mosquito source are given. For the analysis, the mosquitoes were captured or raised in lab (different generations: F2–F30). Different methods were applied to test the ability of the vector mosquito to transmit dengue. A potential transmission was tested via transmission to a host or via an uptake of the virus by the salivary glands of the mosquito. The EIP is given in days.*

Author	Year	Species	Mosquito source	SG	TM	T (°C)	EIP (d)
Blanc	1930	Ae. aegypti	captured in Athens	no	yes	22	9
Blanc	1930	Ae. aegypti	raised in the lab	no	yes	22.5	15
McLean	1974	Ae. aegypti	lab colony[1]	yes	yes	32	6
McLean	1974	Ae. aegypti	lab colony[1]	yes	yes	32	6
McLean	1974	Ae. aegypti	lab colony[1]	yes	yes	27	13
McLean	1974	Ae. aegypti	lab colony[1]	yes	yes	27	13
McLean	1974	Ae. aegypti	lab colony[1]	yes	no	13	35
McLean	1974	Ae. aegypti	lab colony[1]	yes	no	13	35
McLean	1975	Ae. aegypti	lab colony[1]	yes	yes	13	32
McLean	1975	Ae. aegypti	lab colony[1]	yes	no	13	42
McLean	1975	Ae. aegypti	lab colony[1]	yes	yes	21	46
McLean	1975	Ae. aegypti	lab colony[1]	yes	yes	21	32
McLean	1975	Ae. aegypti	lab colony[1]	yes	no	13	42
McLean	1975	Ae. aegypti	lab colony[1]	yes	no	13	7
McLean	1975	Ae. aegypti	lab colony[1]	yes	no	13	7
McLean	1975	Ae. aegypti	lab colony[1]	yes	yes	21	13
McLean	1975	Ae. aegypti	lab colony[1]	yes	no	21	42
McLean	1975	Ae. aegypti	lab colony[1]	yes	no	21	7
Rohani	2009	Ae. aegypti	lab colony (> 30years old)	no	not tested	26	9
Rohani	2009	Ae. aegypti	lab colony (> 30years old)	no	not tested	28	9
Rohani	2009	Ae. aegypti	lab colony (> 30years old)	no	not tested	30	5
Rohani	2009	Ae. aegypti	lab colony (> 30years old)	no	not tested	26	9
Rohani	2009	Ae. aegypti	lab colony (> 30years old)	no	not tested	28	9
Rohani	2009	Ae. aegypti	lab colony (> 30years old)	no	not tested	30	5
Salazar	2007	Ae. aegypti	collected in Mexico (F3-F6, lab)	yes	not tested	28	4
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	no	26	12
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	yes	30	12
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	yes	32	7
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	yes	35	7
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	yes	30	25
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	no	24	18
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	no	26	18
Watts	1987	Ae. aegypti	collected in Bangkok (F2 progeny)	yes	no	30	18
Anderson	2006	Ae. aegypti	McAllen (Texas) strain (F4)	yes	not tested	30	3
Anderson	2006	Ae. aegypti	McAllen (Texas) strain (F4)	yes	not tested	30	4
Anderson	2006	Ae. aegypti	McAllen (Texas) strain (F4)	yes	not tested	30	2
Anderson	2006	Ae. aegypti	McAllen (Texas) strain (F4)	yes	not tested	30	5
Anderson	2006	Ae. aegypti	McAllen (Texas) strain (F4)	yes	not tested	30	9
Anderson	2006	Ae. aegypti	McAllen (Texas) strain (F4)	yes	not tested	30	4
Xiao	2014	Ae. albopictus	Shanghai specimens (> F 30, lab)	yes	not tested	21	10
Xiao	2014	Ae. albopictus	Shanghai specimens (> F 30, lab)	yes	not tested	26	7
Xiao	2014	Ae. albopictus	Shanghai specimens (> F 30, lab)	yes	not tested	31	4
Xiao	2014	Ae. albopictus	Shanghai specimens (> F 30, lab)	yes	not tested	36	4
Vega-Rua	2013	Ae. albopictus	collected in south-east France	yes	not tested	28	9

[1] British Columbia Research Council lab

Declaration of originality

Hereby, I declare that this Master thesis was written by me and that I did not use any other sources and means than specified. This Master thesis was not submitted at any other university for acquiring an academic degree.

Lena Muffler
May 28, 2015, Bayreuth