The Asian Tiger Mosquito *Aedes albopictus* in Switzerland: Biology, Surveillance and Control

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Summary

Background: The Asian tiger mosquito *Aedes albopictus* originates from the tropical and subtropical regions of Southeast Asia, from the islands of the Western Pacific and the Indian Ocean. Over the last century, *A. albopictus* has spread globally. This species is able to form dormant egg stages that survive long periods of dryness and also low temperatures. The eggs are passively dispersed, primarily through the trade with used tyres and plant cuttings. The tiger mosquito is a proven vector for many arboviruses, most notably dengue and chikungunya, with recent outbreaks also in continental Europe. In 2003, *A. albopictus* was spotted for the first time in Switzerland, in the southernmost part of the Canton of Ticino. Since then the local authorities have continued its surveillance and control. The control programme mainly includes larval source reduction alongside larvicidal applications. Despite these efforts, mosquito densities have increased over the last decade, casting doubts on the effectiveness of such larval control programmes. The Italian communities just across the border lack such a surveillance and intervention programme providing the possibility to compare an intervention versus a non-intervention area side by side.

Objectives: The overall aim of this work was to study the biology of the *A. albopictus* population in Switzerland to better understand the risk of local vector-borne disease transmissions, by reviewing the effect of existing control measures in the Canton of Ticino. Four specific objectives were pursued in this PhD: (i) to compare the spatial and temporal distribution of *A. albopictus* in Ticino (intervention) with its distribution in the neighbouring Italian communities (non-intervention) just across the national border, evaluating the impact of the Ticino control programme, (ii) to assess the insecticide susceptibility status of the *A. albopictus* population in Swiss-Italian border region, (iii) to investigate host preferences of *A. albopictus*, (iv) to assess the vector competence of the local *A. albopictus* population for dengue virus (DENV) transmission und local conditions.

Research partnership: This PhD project was carried out within the frame of the Brazilian Swiss Joint Research Programme (BSJRP) and was a collaboration of the Centro de Pesquisa Aggeu Magalhães-FIOCRUZ in Recife, Brazil, the cantonal mosquito working group (GLZ) in Ticino, Switzerland and the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland.

Methods: Using specialised traps that collect eggs from egg laying female mosquitoes and a randomised sampling scheme, the seasonal and spatial abundance of *A. albopictus* was examined and compared between the intervention area (Ticino, Switzerland) and the adjacent non-intervention area (Lombardy, Italy) both in sylvatic and urban habitats in 2012 and 2013. *A. albopictus* colonies from field-caught mosquitoes were established in the laboratory and insecticide susceptibility assays performed according to the guidelines of the World Health Organization (WHO). Blood meals of field-caught *A. albopictus* females were identified using polymerase chain reaction (PCR) and matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) methods. In infection studies it was also investigated whether field-caught *A. albopictus* are competent to transmit dengue virus (DENV) serotypes DENV-1, DENV-2 and DENV-3.

Results: The results show that the relative *A. albopictus* density is 2.26 times higher on the Italian side of the border as compared to Ticino. In this study another invasive mosquito species, *A. koreicus*, was detected and desribed for the first time in Switzerland. Encouragingly, *A. albopictus* collected both in Italy and Ticino is still fully susceptible to the insecticides employed as well as several alternatives. Field-caught *A. albopictus* females showed a clear preference for mammalian blood, while blood meals were predominantly of human origin, followed by cows and to a lesser extent chicken and sheep. Preliminary data from infection studies suggest that the Swiss *A. albopictus* population may be competent to transmit dengue viruses under local Swiss climatic conditions. Unfortunately, the experiments proofed challenging and are not yet conclusive.

Conclusions: Though alternative explanations are also valid, the results support the hypothesis that the intervention programme in Southern Switzerland, targeting larval *A. albopictus*, does have a significant impact. At the same time the data also suggest that current larval interventions fall short in gaining full control over the mosquito, calling for the evaluation of additional, or alternative, approaches. Ideally, these should also consider inclusion of the neighbouring Italian communities in the surveillance and control efforts. Most likely local transmissions of diseases by *A. albopictus* are also possible in Switzerland.

Within the frame of the national surveillance programme in Switzerland positive ovitraps were also found in the northern part of Switzerland. Both the number of positive ovitraps and the number of eggs per trap increased from 2013 to 2014. Most

likely the observed cases represent only single introduction events. The fact that egg numbers are increasing in Switzerland and that *A. albopictus* has also been detected in southern Germany at several motorway stations along the German extension (A5) of the motorway A2 near Basel is remarkable. Data suggest the Gotthard route to be an important route for the passive spread of *A. albopictus* via ground traffic from Italy to northern Europe.

For public Health reasons it is advisable to observe the spread and establishment of invasive mosquito species in Switzerland. Moreover there is a pressing need for the development of an action plan at both the national and regional level. A communication network need to be set up with clearly assigned responsibilities and tasks.

Zusammenfassung

Hintergrund: Die asiatische Tigermücke *Aedes albopictus* stammt ursprünglich aus den tropischen und subtropischen Regionen Südostasiens, von den Inseln im westlichen Pazifik und Indischen Ozean. Im Verlaufe des letzten Jahrhunderts hat sich *A. albopictus* global ausgebreitet. Diese Spezies kann sogenannte dormante Eierstadien bilden, die längere Trockenperioden und niedrige Temperaturen überleben. Solche Eier werden dann passiv verbreitet, hauptsächlich durch den Handel mit gebrauchten Autoreifen und Pflanzen. Die Tigermücke ist eine bekannte Überträgerin von vielen Arboviren, unter anderem das Dengue- und Chikungunyavirus, und war in näherer Vergangenheit für mehrere Krankheitsfälle in Europa verantwortlich. Im Jahr 2003 wurde *A. albopictus* zum ersten Mal in der Schweiz nachgewiesen, im südlichsten Kanton Tessin. Seit diesem Zeitpunkt wird die Tigermücke im Kanton kontinuierlich überwacht und bekämpft. Das Kontrollprogramm basiert hauptsächlich darauf, mögliche Brutstätten entweder zu eliminieren oder mit Insektiziden zu behandeln, die gegen Mückenlarven wirken. Trotz all dieser Bemühungen hat sowohl die Verbreitung als auch die Dichte der Mücken im letzten Jahrzehnt ständig zugenommen, was Zweifel daran aufkommen lässt, ob solche Kontrollprogramme, die hauptsächlich auf Mückenlarven zielen, wirklich effektiv sind. In den italienischen Gemeinden auf der anderen Seite der Nationalgrenze findet keine Überwachung oder Bekämpfung der Tigermücke statt. Dies ermöglichte einen Vergleich zwischen einem Gebiet mit Interventionen gegen *A. albopictus* und einem ohne.

Ziele: Das Hauptziel dieser Arbeit war, die Biologie der Tigermückenpopulation in der Schweiz zu studieren und das Risiko für die Übertragung von Krankheiten besser zu verstehen. Hierfür wurde unter anderem die existierende Bekämpfungsstrategie im Tessin auf ihren Effekt hin untersucht. Vier spezifische Ziele wurden in dieser Doktorarbeit verfolgt: (i) ein Vergleich der räumlichen und zeitlichen Verbreitung von *A. albopictus* zwischen dem Kanton Tessin, wo die Mücke bekämpft wird, und italienischen Gemeinden auf der anderen Seite der Grenze, wo keine Bekämpfung stattfindet, (ii) die *A. albopictus* population in der schweiz-italienischen Grenzregion auf Resistenzen gegen Insektizide zu untersuchen, (iii) die Wirtspräferenz von *A. albopictus* zu untersuchen, (iv) die Vektorkompetenz der lokalen *A. albopictus* population für Dengueviren zu überprüfen.

Forschungszusammenarbeit: Diese Doktorarbeit wurde im Rahmen des Brazilian Swiss Joint Research Programme (BSJRP) durchgeführt und war eine Zusammenarbeit

des Centro de Pesquisa Aggeu Magalhães-FIOCRUZ in Recife, Brasilien, der kantonalen Arbeitsgruppe zur Mückenbekämpfung (GLZ) im Tessin, Schweiz, und dem Schweizerischen Tropen- und Public Health-Institut in Basel, Schweiz.

Methoden: Um die räumliche und saisonale Verbreitung der Tigermücke im Interventionsgebiet mit jener in den italienischen Gemeinden zu vergleichen und zu messen, wurden während den Sommermonaten 2012 und 2013 spezielle Fallen eingesetzt, in denen Mückenweibchen ihre Eier ablegen. Diese Fallen wurden an zufällig bestimmten Orten aufgestellt, sowohl in urbanen als auch bewaldeten Gebieten. Im Feld gesammelte Mückeneier wurden aufgezogen und dazu verwendet, Laborkolonien zu etablieren. Den Richtlinien der Welt-Gesundheitsorganisation (WHO) folgend, wurden diese Mücken experimentell auf Insektizidresistenzen untersucht. Blutmahlzeiten von im Feld gefangenen Mückenweibchen wurden mithilfe der Polymerasen-Kettenreaktion (PCR) und matrix-unterstützter Laser-Desorption/Ionisation time- of-flight Massenspektrometrie (MALDI-TOF MS) untersucht und identifiziert. Infektionsstudien wurden durchgeführt um festzustellen, ob die *A. albopictus* population im Tessin die Denguevirus Serotypen 1, 2 und 3 übertragen kann.

Resultate: Die Resultate zeigen, dass die relative Dichte von *A. albopictus* auf der italienischen Seite der Grenze 2.26-fach höher ist als im Tessin. Während den Feldarbeiten dieser Studie wurde eine andere invasive Mückenspezies entdeckt, *A. koreicus*. Dies ist der erste Nachweis dieser Art in der Schweiz. Glücklicherweise zeigten sich die im Feld gesammelten Mücken empfänglich gegenüber den momentan eingesetzten Insektiziden und anderen, alternativ getesteten Giftstoffen. Es zeigte sich eine klare Wirtspräferenz für Säugetiere. Blut von Menschen und Kühen war klar dominierend in den analysierten Blutmahlzeiten, gefolgt von Hühner- und Schafsblut. In ersten Infektionsexperimenten bestätigte sich, dass die schweizerische *A. albopictus*- Population Dengueviren übertragen kann. Leider unterlagen diese Studien grossen Herausforderungen und können noch nicht als konklusiv betrachtet werden..

Schlussfolgerungen: Auch wenn andere Erklärungen nicht ausgeschlossen werden können, wird die Hypothese, dass die Bekämpfungsmassnahmen gegen *A. albopictus* im Tessin einen signifikanten Effekt haben, durch die Resultate unterstützt. Gleichzeitig zeigen die Daten auch, dass die momentan getroffenen Massnahmen nicht ausreichen, um die Situation vollständig unter Kontrolle zu halten. Zusätzliche und alternative Vorgehensweisen müssen evaluiert werden. Idealerweise müssten die italienischen

Gemeinden in das Kontrollprogramm miteinbezogen werden. Übertragungen von Krankheiten durch die Tigermücke sind in der Schweiz potenziell möglich.

Im Rahmen des schweizerischen Überwachungsprogramms wurde die Tigermücke auch nördlich der schweizer Alpen nachgewiesen. Sowohl die Anzahl positiver Fallen als auch die Anzahl Eier pro Falle haben zwischen den Jahren 2013 und 2014 zugenommen. Höchstwahrscheinlich handelt es sich bei diesen Funden um Einschleppungen von einzelnen Mücken. Die Tatsache, dass *A. albopictus* an der nördlich von Basel gelegenen deutschen Autobahn gefunden wurde und dass die Eierzahlen tendenziell zunehmen, sind auffallend. Die Daten lassen vermuten, dass die Gotthard-Route eine wichtige Achse darstellt für die passive Verbreitung von *A. albopictus* von Italien in das nördliche Europa.

Aus gesundheitspolitischer Sicht ist es ratsam, die weitere Verbreitung und Etablierung invasiver Mückenarten in der Schweiz genau zu beobachten. Ausserdem werden dringend Aktionspläne benötigt, sowohl auf regionaler als auch nationaler Stufe. Ein Kommunikationsnetzwerk muss geschaffen werden, in dem Verantwortung and Aufgaben definiert und zugewiesen werden.

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Abbrevations

BG	Biogents
BSJRP	Brazilian-Swiss Joint Research Programme
Bti	Bacillus thuringiensis israeliensis
CBD	Convention on Biological Diversity
CDC	Centres for Disease Control
CHKV	Chikungunya Virus
DDT	Dichlordiphenyltrichlorethan
DENV	Dengue Virus
ECDC	European Centres for Disease Control
EI	Inhibition Concentrations
EMCA	European Mosquito Control Association
E-SOVE	European Society for Vector Ecology
FOEN	Federal Office of Environment
FSVO	Federal Food Safety and Veterinary Office
GDD	Growing Degree Days
GIS	Geographic Information System
GLZ	Gruppo Lavoro Zanzare
GPS	Global Positioning System
IGR	Insect Growth Regulator
IMS	Invasive mosquito species
IUCN	International Union for Conversation of Nature
LD	Lethal Dosage
MALDI-TOF MS	Matrix-Assisted Laser-Desorption/Ionization Time-Of-Flight Mass-Spectrometry
MBD	Mosquito Borne Disease

OP	Organophosphate
PCR	Polymerase Chain Reaction
PEAa	National Programme in Brazil for eradicating *A. aegypti*
PNCD	National Programme for Dengue Control
RMR	Recife Metropolitan Region
SKEW	Swiss Commission for Wild Plant Conservation
SUPSA	Scuola universitaria professionale della Svizzera italiana
SVEG	Swiss Vector Entomology Group
Swiss TPH	Swiss Tropical and Public Health Institute
UK	United Kingdom
USA	United States of America
WGS	World Geodetic System
WHO	World Health Organisation
ZINB	Zero-Inflated Negative Binominal Model

Chapter 1

Introduction

1. Introduction

In the following introduction sections detailed information is provided on general characteristics of invasive animal and plant species, the biology and distribution of invasive mosquito species (IMS) in Europe, and on the current situation of *Aedes albopictus*, including surveillance and control, both in Italy and Switzerland. The scientific hypotheses of this dissertation are formulated in the last introduction chapter and are put in context to the provided background information.

1.1 Introduction on invasive species

According to the Convention on Biological Diversity (CBD) [1] an alien organism is defined as being a species, subspecies or lower taxon, introduced outside its natural past or present distribution, including any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce. Alien species became a raising problem if they are invasive, meaning they are spreading, or have the potential to spread in an area, at the expense of native species. Invasive species can damage biodiversity in various ways: native species are displaced because the invasive species is more competitive, hybridisation with native populations takes place, the functioning of native ecosystems gets disrupted by altering ecological factors, and diseases and parasites are transmitted that are not found in the native species. Invasive species often have adversely effects on human society. First of all they can cause health problems in many ways. The pollen of the invasive plant species *Ambrosia artemisiifolia* for example, is an allergen to many people with hay fever and often causes severe asthma [2]. In Switzerland, approximately 20% of the public is affected [3]. The seeds of this plant remain germinal for 40 years. It was introduced to Europe with contaminated seeds for agriculture and bird seeds from northern America.

Sometimes invasive species have the potential to serve as vectors of pathogens causing disease in humans and animals. Here, especially mosquitoes are of concern. Globally, over one million people die from mosquito-borne diseases every year and hundreds of millions experience pain and suffering from illnesses transmitted by mosquitoes [4]. Also ticks and fleas transmit parasites, viruses or bacteria between people or between animals or people. According to the Centres for Disease Control (CDC) vector-borne diseases

account for 17% of the estimated global burden of all infectious diseases. Global trade, rapid international travel, and environmental changes such as climate change and urbanisation are causing vectors and vector-borne diseases to spread globally.

Non-native invasive species can also damage the economy, especially agriculture. Beside causing health problems, the above mentioned *A. artemisiifolia* plant is also highly competitive and causes low yields in sunflowers, pea and soy plants [3]. There are some invasive plant species that complicate the maintenance of infrastructure like railway lines, roads and shorelines. Another example of an invasive alien species is the Asian ladybeetle *Harmonia axyridis*. This species is a well-known predator of aphids and was therefore used as a biological control agent in agriculture. By the end of the 20st century it was introduced to the United States and later on to Europe. In Europe it mainly served as a tool for the control of aphids in hop gardens and greenhouses [5]. In the following years the Asian ladybeetle expanded and established in many areas. First being an effective control agent it turned to a pest. Besides replacing native ladybeetle species it also impacts vine and fruit production. Together with the grapes and fruits they end up in the mesh or must and negatively affect the taste [5, 6]. The control of *H. axyridis* is very difficult since it has only few natural enemies. In many European countries the trade, rearing and dispersal of this species is now prohibited.

Invasive animal and plant species are increasingly attracting attention in horticulture and agriculture sectors. According to the International Union for Conservation of Nature (IUCN), the threat to biodiversity from invasive species is only second after habitat destruction. The establishment of non-indigenous species has been implicated as the causal mechanism for 20% of animal extinctions globally and as a secondary contributor to an additional 34% of animal extinctions [7, 8].

A successful invasion process requires transport and arrival of an alien species to and within a new area. The species needs to survive and establish a stable population in the new environment. A successful transition between those different stages depends on three factors: i) propagule pressure (i.e. number of introduced individuals), ii) physiological tolerance to environmental conditions, and iii) integration into the biological community [9, 10]. From a global perspective, most invasion opportunities are not successful [9, 11].

In Switzerland invasive species management is a task shared between the federal and cantonal authorities. Federal authorities are responsible for giving concrete form to

regulations concerning the management of invasive alien species, and for coordinating management efforts at the intercantonal, federal and international level [12]. They raise awareness, provide information and promote cantonal enforcement. Also research is commissioned, i.e. the development of new criteria and methods to facilitate enforcement in the areas of early detection, monitoring, control and outcome evaluation. The Ordinance on the Release of Organisms (Freisetzungsverordnung) of the environmental law demands a duty of care, self-regulation for placing on the market, an obligation to inform and instruct recipients in dealing with invasive organisms in the environment [12]. According to the Federal Office of Environment (FOEN) about 10% of the Swiss flora are currently considered alien species of which another 10% are invasive. Since they potentially cause substantial damage it is necessary to have an updated monitoring and to stop their expansion wherever possible. Concerning invasive plant species the Swiss commission for wild plant conservation (SKEW) manages an information centre on invasive alien plants (www.infoflora.ch). There is a Black List with invasive alien plant species that adversely affect biodiversity, public health or the economy in Switzerland. Invasive plant species with the potential to cause damage are also on a watch list, as their spread needs to be monitored.

Invasive animal species are often introduced by animal enthusiasts. They escape from captivity or spread spontaneously (i.e. grey squirrel, Asian clam). In Switzerland, the Federal Office of Environment (FOEN) and the Federal Food Safety and Veterinary Office (FSVO) are responsible for regulating imports and exports of non-native animal species which may be kept in captivity but not released into the wild.

1.2 Invasive mosquitoes in Europe

Invasive mosquito species (IMS) are defined by their ability to colonise new territories and to cause or to be likely to cause harm to the economy, environment, or human and animal health [13]. Currently there are five IMS known to be established in Europe, all belonging to the *Aedes* genus: *A. albopictus, A. aegypti, A. japonicus, A. atropalpus* and *A. koreicus* [13]. Human activity, especially the global movement of goods, has led to the passive dispersion of mosquito species previously confined to specific regions [14]. Additionally, several studies have previously highlighted the increasing climatic suitability for IMS in Europe as a consequence of climate change [15–18]. Once

established in a new territory, the success of IMS in reproducing and spreading depends on a complex range of intrinsic population factors, like longevity and host-seeking behaviour, and extrinsic parameters like climate, human population movements, travel and trade [14].

Most invasive mosquito species that successfully invade new territories are able to produce dormant egg stages. These eggs are normally formed towards the end of the summer season when days become shorter and are more resistant to desiccation and cold temperatures. Desiccation-resistant eggs are strongly associated with a mosquito becoming an introduced non-native species, most likely because this favours its successful passive distribution [19]. Among invasive and non-native mosquito species it is also common to breed in small man-made containers, tree holes, bromeliads or rock pools. As a consequence these mosquito species establish in more urban environments where they increasingly come in contact with humans. Especially day-active mosquitoes cause noticeable biting nuisance and affect life quality when they appear in high densities. In temperate regions mosquito borne diseases (MBDs) represent a lower burden compared to tropical regions where they substantially impact the countries` socio-economic development. In Europe, though to a lesser extent, both endemic and epidemic autochthonous MBDs have also occurred [15, 20–25]. Concern is now rising as both vectors and pathogens are increasingly being introduced through international travel and trade. Their occurrence is often associated with changes in ecosystems, human behaviour, and climate [26]. According Petrić et al. [27] an estimate 45% of the total European human population is exposed to the risk of IMS and pathogens they could transmit.

Considering the vector potential and the ability of colonising a wide range of different environments, *A. albopictus* and *A. aegypti* are currently the most important invasive mosquito species in Europe. Under experimental conditions, *A. albopictus* is a competent vector for 22 arboviruses, including all four dengue serotypes, yellow fever, chikungunya and Ross River virus [28]. Same is true for *A. aegypti*. The Asian bush mosquito *A. japonicus* has a lower significance since it was only shown to be a competent vector in the lab but not yet in the field [29]. *A. albopictus* and *A. aegypti* are also known to be efficient vectors in the field for a wide range of viruses [28, 30]. These two species were responsible for historic and recent epidemics/cases of MBDs in Europe. The first more recent outbreak of a mosquito-borne disease in Europe linked to

autochthonous transmissions of invasive mosquito species occurred in Ravenna, Italy in 2007. More than 200 confirmed cases of chikungunya fever were reported and one person died [20]. The rapid spread of the infection demonstrated the efficiency of local *A. albopictus* populations to transmit chikungunya virus (CHIKV). More recently, between August and September 2010, even autochthonous dengue cases were reported from Croatia and metropolitan France with *A. albopictus* deemed responsible for its transmission [21, 22]. In the same year, two people became also infected with the chikungunya virus in Fréjus, France [23]. Then additional cases were reported from mainland Portugal [31] and again from Southern France [24] in 2012 and 2013, respectively, and most recently in 2014, another four autochthonous dengue- and eleven chikungunya cases have been detected in France [25]. *A. albopictus* is also of veterinary significance because it is a competent vector for *Dirofilaria immitis*, a nematode that causes dirofilariosis in dogs [28]. These outbreaks clearly show that Europe is vulnerable for the transmission of "tropical" arboviruses, particularly in regions where *A. albopictus* and *A. aegypti* are present. In Central Europe *A. albopictus* has a higher importance (Fig. 1.1) since *A. aegypti* is currently only present on Madeira, few countries around the Black Sea (Southern Russia, Abkhazia, Georgia) and the Netherlands [32], (Fig. 1.2).

An early detection of invasive mosquito species enables appropriate and timely response measures and subsequent prevention of MBDs [33]. In areas where invasive mosquito species become or could become established it is very important to implement surveillance programmes to follow up their abundance and spread in order to assess the risk of pathogen transmissions to humans [34]. The European Centre for Disease Control (ECDC) developed guidelines for the surveillance of invasive mosquitoes in Europe in order to harmonise surveillance methods and strategies and also to encourage member states to collect appropriate data on IMS in the field [34, 35]. Various European countries have started mosquito monitoring and surveillance programmes, sometimes also including screening of mosquitoes for the presence of pathogens [36]. Usually such surveillance programmes rely on the use of mosquito traps for determining the occurrence and spatial-temporal distribution of mosquitoes. However, managing a country-wide network of mosquito traps needs expertise is expensive, time and labour-consuming. To save resources, some countries rely on passive surveillance using other data sources. For example Germany, Spain and France involve the general public in that

citizens are encouraged to report mosquito findings. Such strategies help to collect additional data, minimising the effort and to alleviating costs [37]. Including observations by the interested public is known as citizen science and has become increasingly popular over the last years [38–40]. Especially since the presence of a nuisance species, native or non-native, is usually perceived for the first time by local inhabitants [41, 42]. New developments in information science are crucial for the success of such initiatives. Nowadays, data informatics, graphical user interfaces, and geographic information system-based web applications can be ported to smartphones and other hand-held devices [38]. This helps to establish efficient communication channels between the community, scientists and authorities and may therefore contribute to the early detection of changes in the mosquito fauna. There are examples of successful citizen-based passive surveillance programmes are the “Mückenatlas” in Germany [43]), the “Mosquito Watch” in the UK [44], the “Muggeradar” in the Netherlands [45], the “Atrapa el tigre” in Spain [46], the “iMoustique” in France [47] and the “Mosquito WEB” in Portugal [48].

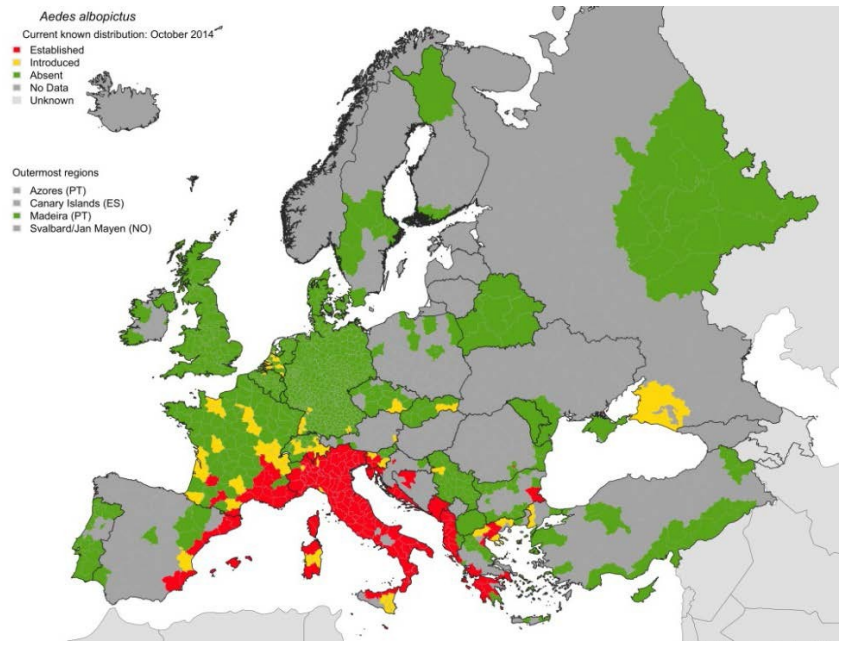

Figure 1.1: Currently known distribution of *Aedes albopictus* by October 2014. Source: ECDC-EFSA 2014/VECTORNET [49] (green: absent, dark grey: no data, light grey: unknown, red: established).

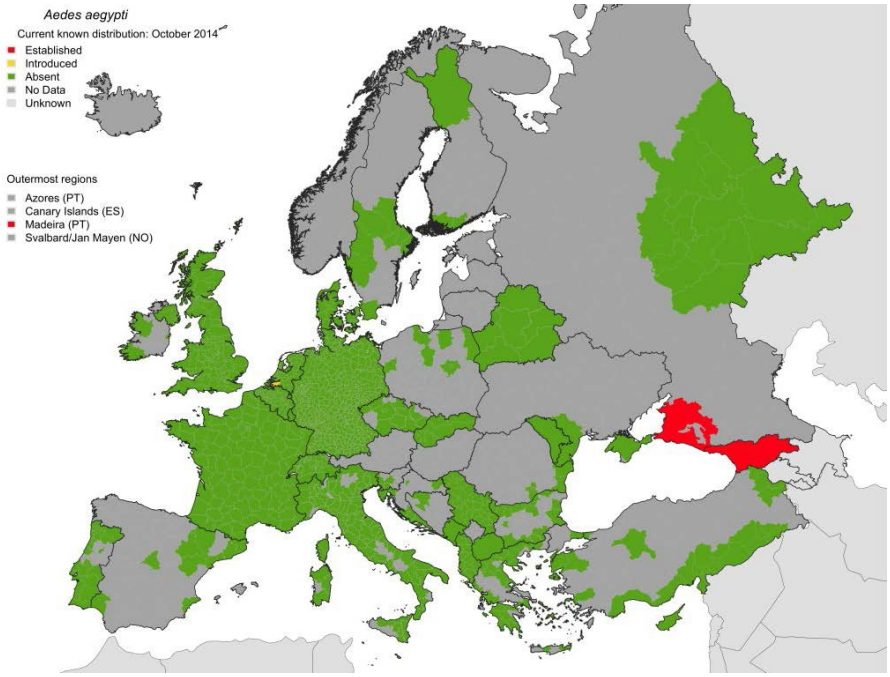

Figure 1.2: Current known distribution of *Aedes aegypti* by October 2014. Source: ECDC-EFSA 2014/VECTORNET [49] (green: absent, dark grey: no data, light grey: unknown, red: established).

1.3 Introduction on *Aedes albopictus* in Italy

The Asian tiger mosquito *A. albopictus* has been reported from 20 European countries, among which Italy is the most heavily infested [32]. The first detection of adult Asian tiger mosquitoes in Italy occurred in Genova in 1990. Since then it spread quickly across the whole country. The most important foci of colonisation developed in the north-eastern regions (Veneto, Friuli Venezia Giulia, Fig. 1.3), along the Adriatic coast as well as in the inner lands (i.e. Garda Lake or Euganean Hills) [50]. In these regions, *A. albopictus* finds ideal environmental conditions to proliferate and to extend its seasonal activity, mainly because of mild temperatures throughout the year.

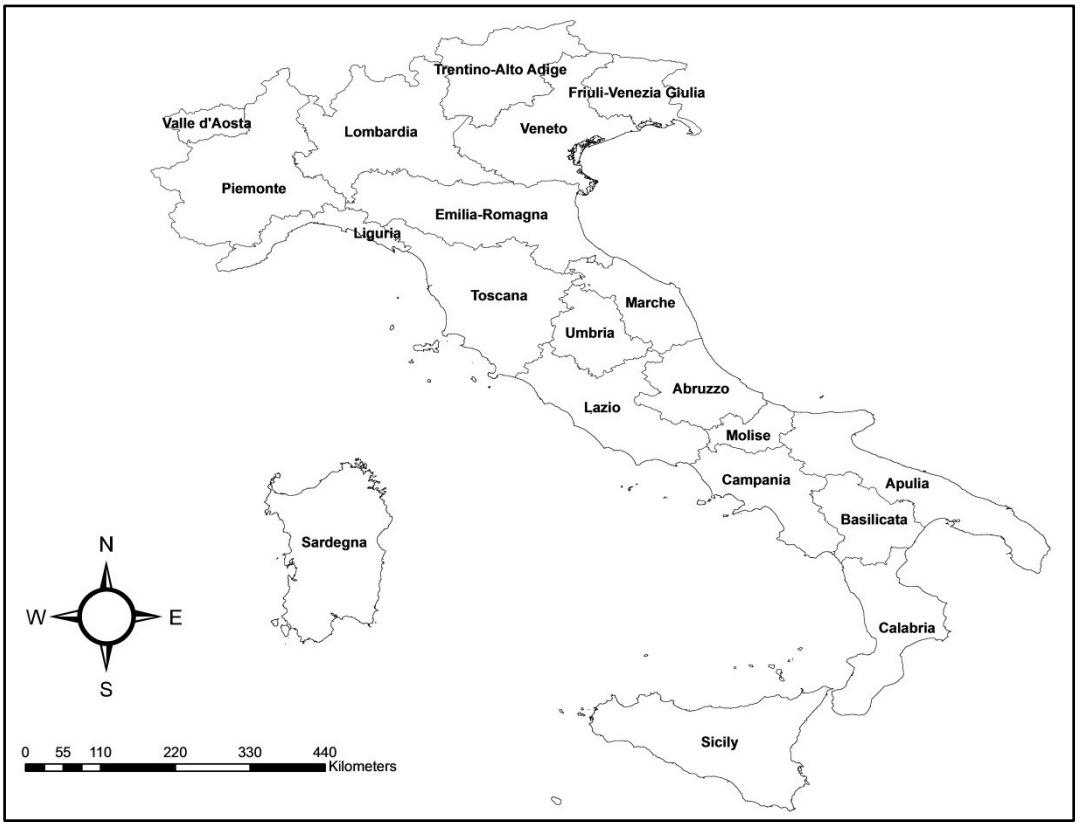

Figure 1.3: Administrative regions in Italy.

Romi et al. [50] assume that *A. albopictus* populations has been introduced to Italy on different occasions and to different areas, while all introductions can be traced back to the South of the United States of America (i.e. Atlanta, Georgia). The mosquitoes were transported in containers with used tyres. Most probably the majority of the imported *A. albopictus* populations arrived in Italy after a long period of "acclimatisation" in the USA in areas north of their natural distribution area, because egg diapause needs to be induced by a temperate climate with short photoperiods and low temperatures. Two major tyre recycling companies are located in the periphery of Padova and Bologna in the Veneto and Emilia Romagna region. They imported scrap tyres directly from the USA, having allowed for a quick spread of the mosquito across Italy throughout internal trade of tyres to smaller companies. Four years after the first entry of *A. albopictus* in Italy, almost all new colonisation foci were located in the vicinity of tyre deposits. In the following years, *A. albopictus* was further distributed by other kinds of passive transport. This species is also known to spontaneously enter cars, lorries and trains. Nowadays, scattered foci of *A. albopictus* are reported from all Italian regions, except

Valle d`Aosta. The mosquito is present in 82 out of 107 provinces, from coastal plains to inner lands, up to 600 metres altitude [50].

In 2008, Talbalaghi [51] criticised that there is a lack of awareness in Italy regarding the risk of vector borne diseases, even though the Italian Ministry of Health has recommended that Public Health services improve arbovirus surveillance [52]. With the exception of a few isolated cases of effective mosquito control management, control programmes are driven by the commercial sector, mainly by insecticide producing companies. Of course this is not an ideal situation and can lead to conflicts of interests. Approximately 900 different companies are involved and have almost become the main authority on mosquito control. Talbalaghi also stated that there is an urgent need for more permanent, reliable and unbiased approaches for vector control. Moreover, national and regional legislation and guidelines for the surveillance and control of vectors are needed. Currently, every municipality is responsible for its own territory, as well as the population being responsible for their own properties. Only few regions, i.e. Piedmont, are carrying out coordinated mosquito control actions. Unfortunately, the success of these efforts are not sustainable as long the neighbouring Lombardy region lacks a control programme and serves as a continuous "source" of *A. albopictus*. Also the province of Rome has implemented a surveillance and control program for *A. albopictus*, funded by the city council of Rome and coordinated by the Istituto Superiore di Sanità. There, the mosquito is present across the whole urban area and in the majority of towns belonging to the province of Rome. It is the first example in Europe of an urban area extensively colonised by the tiger mosquito, affecting hundreds of thousands of people [53].

With regards to CHKV, national coordination of regional activities by the Ministry of Health is not clear. Fortunately, Emilia Romagna was able to effectively manage the chikungunya outbreak in 2007 [20]. This was possible because the administration was equipped with appropriately skilled staff that could handle the situation. However, this is not the case in many other regions of Italy and is therefore an issue of great concern [51]. Gavaudan et al. [52] stated that pest control systems should be performed by different interested parties to ensure reliability. If both surveillance and control are well performed and managed, the risk of arboviral transmissions can be reduced significantly. It is necessary to reach this level of organisation in the major urban areas of the Italian peninsula since mosquito infestations are often largely unpredictable,

posing a high risk for epidemics. Public health scientists as well as entomologists, clinicians or epidemiologists need to push the time table for the implementation of existing techniques for mosquito surveillance and control. In addition, it is particularly important to communicate the benefits and knowhow of such methods to politicians and administrators.

From a Swiss perspective, the implementation of mosquito control programmes in Italy, especially in the border areas to Switzerland, is of course highly desirable and important in terms of reducing the number of "border-crossing" *A. albopictus* mosquitoes.

1.4 Introduction on *Aedes albopictus* in Switzerland

After the detection of *A. albopictus* in Italy and after its expansion to many parts of the country, including the border region south of Switzerland [54], a surveillance programme was put in place in the Canton of Ticino, southern Switzerland, by the local authorities in 2000. A working group, the Gruppo Lavoro Zanzare (GLZ), was founded to coordinate surveillance and later on also the control of *A. albopictus* in the Canton. In 2003 first eggs were detected in ovitraps [55]. Between 2003 and 2006, the monitoring effort was gradually intensified. Assumingly, *A. albopictus* could profit from the intense traffic circulation of people and merchandise in the border area to Italy [56]. Therefore GLZ focused the surveillance on locations situated along the main South-North traffic axis, the A2 motorway, such as popular shopping centres and service stations. The monitoring was based on ovitraps.

In the following years the estimated tiger mosquito density was still low, suggesting that individual adult mosquitoes had been sporadically introduced from Italy but had not yet established a sustained population in Ticino. In 2007, the situation changed significantly, when a dramatic increase of positive mosquito traps in Chiasso, just across the Swiss-Italian border, was recorded, indicating that a local mosquito population had now established [57]. In the following, surveillance in Ticino was expanded and control strategies implemented [56]. In 2007, the monitoring system consisted of over 70 sampling sites with a total of 300 oviposition traps. The traps were set within the communities and along the Ticino motorway at parking and resting areas. The GLZ uses ovitraps that consist of a water-filled black cylinder with a piece of wood

on which female mosquitoes can lay their eggs (Fig. 1.4). Weekly control visits to all traps were conducted between April and November. As soon as eggs were detected, the surrounding vegetation within a perimeter of approximately 100 metres was sprayed with permethrin against adult mosquitoes. Stagnant water was treated with *Bacillus thuringiensis israelensis (Bti)* and in some cases with diflubenzuron to control the larval stages [58, 59].

During the last years, this network has been continuously expanded and further adapted. Today, more than 1,200 ovitraps are used. The traps are checked biweekly and the number of positive traps serves as an indicator whether and where insecticide or larvicide use is necessary. In addition, information campaigns are carried out to raise public awareness in order to eliminate potential breeding sites from private grounds and to sensitise people on the occurrence of *A. albopictus* (see flyer, appendix 8.3). Despite the efforts *A. albopictus* densities have increased in the Canton of Ticino over the last years [60]. Egg numbers as well as the proportion of positive ovitraps have risen in all regions under surveillance.

Figure 1.4: Ovitrap in Ticino, Switzerland. Mosquitoes are attracted by the water in the container and deposit their eggs on the wooden slat. The *Bti* granules in the water are lethal to mosquito larvae and prevent adult emergence.

Since *A. albopictus* is now well established in the Canton of Ticino it is important to evaluate, if it could also further expand to more northern parts of Switzerland. From different studies it is known that the spatial distribution and colonisation of new areas

by *A. albopictus* depend on several meterological parameters, such as winter and summer temperatures and precipitation patterns. *A. albopictus* is known to be sensitive to low temperatures [32, 61, 62]. January mean temperatures have been implicated as a threshold for population stability and likelihood of survival [17, 63, 64]. According to Neteler et al. [63], suitability of an area for the survival of *A. albopictus* populations can also be determinated by using the growing degree days (GDD). GDD are defined as the degrees exceeding a given threshold (11°C for *A. albopictus*) accumulated for all days in a given year [61]. In addition to temperature, *A. albopictus* is also very sensitive to aridity. Five hundred mm precipitation per year is considered the minimum threshold value [17]. In 2013, Neteler et al. [65] did a study on the identification of most suitable areas in Switzerland for the invasion and establishment of *A. albopictus*. They considered both current (Fig. 1.5) and predicted future (Fig. 1.6) climate conditions. In order to assess potential distribution areas, two different climate change scenarios for Switzerland for the periods 2020-2049 and 2045-2074 were considered.

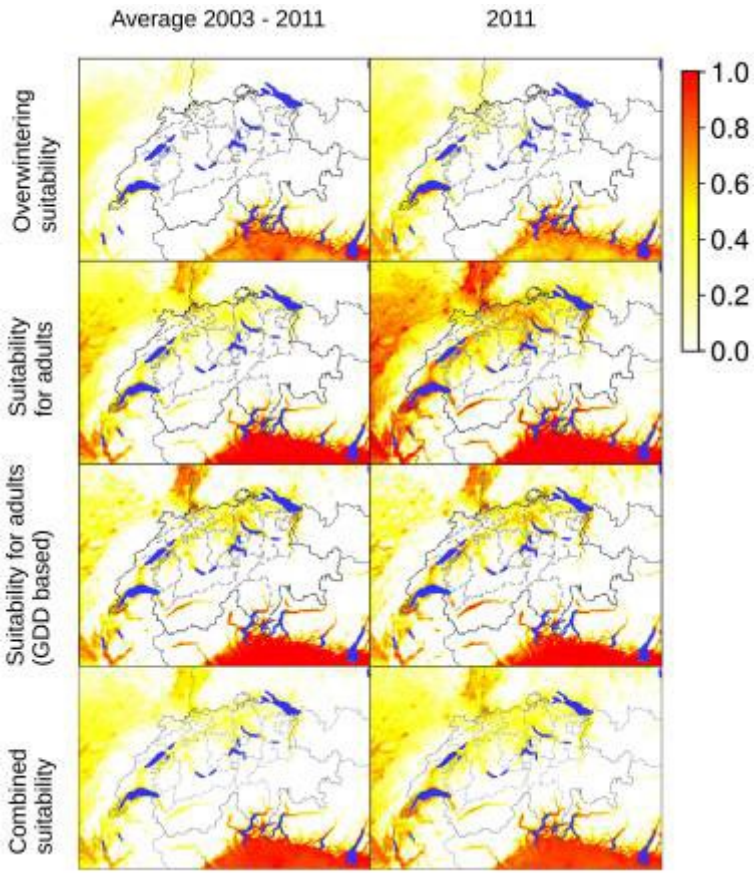

Figure 1.5: The maps in the first column are based on averages for the years 2003–2011; the maps in the second column are based on temperature values for 2011 only. The colouring scheme shows the gradient of suitability, from 0.0 (unsuitable areas: white coloured) to 1.0 (highly suitable areas: red coloured). GDD: growing degree days filtered for days above 11°C [65].

According to Fig. 5, in northern Switzerland, parts of the Rhine valley, both upstream and downstream of Lake Constance, as well as areas surrounding Lake Neuchatel appear to be suitable for adult *A. albopictus*. However, current winter temperatures are too cold to allow survival of eggs. According to climate models, also the region of Basel seems to be suitable for adult tiger mosquitoes. Also the overwintering of diapausing eggs is possible, even though the gradient of suitability is quite low (Fig. 1.5). Highly suitable areas for adult mosquitoes are the Canton of Geneva and areas surrounding Lake Leman, the main Rhone valley in the Canton of Valais and the Canton of Ticino, including the entire southern part, the region of Locarno, and the district of Riviera. According to the models based on extrapolated future climatic scenario suitable regions for *A. albopictus* in Switzerland will further expand (Fig. 1.6). Depending on the season and region considered, the medium estimates of the A1B emission scenario foresee a warming of 0.9–1.4°C by the period 2020–2049, 2.0–2.9°C by 2045–2074, and 2.7–4.1°C by 2070–2099, as well as a decrease in summer mean precipitation by 10–17% by 2045–2074 and 18–24% by 2070–2099. The A2 emission scenario also includes an assumption about economic growth and technological progress, but at a slower rate and more heterogeneously. It predicts an increase in the seasonal mean temperature of 3.2–4.8°C by the end of the century (period 2070–2099) and a reduction in summer mean precipitations by 21–28%, depending on region.

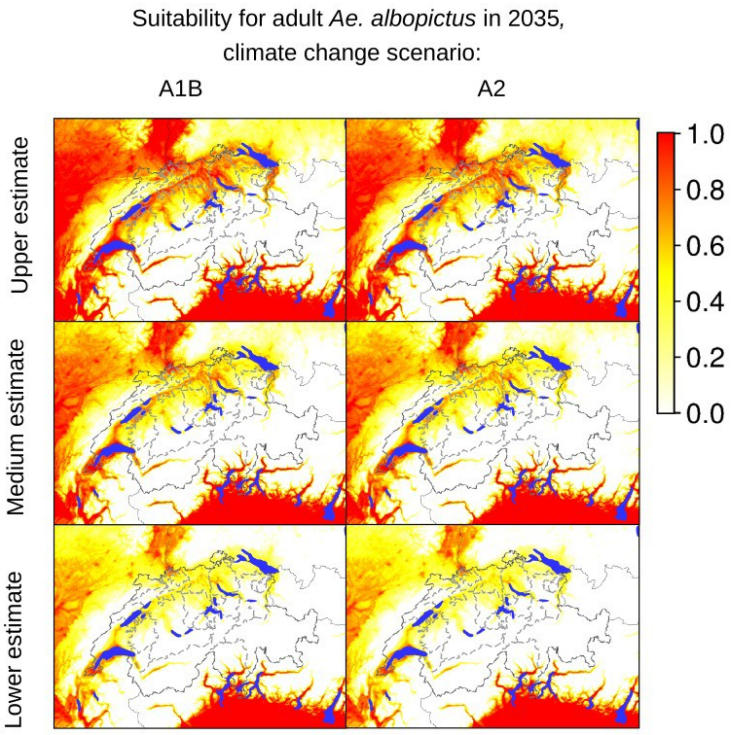

Figure 1.6: The maps in the first column are based on temperature estimates for the climate changes scenario A1B, the maps in the second column are based on temperature estimates for the climate changes scenario A2. The colouring scheme shows the gradient of suitability, from 0.0 (unsuitable areas: white coloured) to 1.0 (highly suitable areas: red coloured) [65].

These climatic models show that an establishment of *A. albopictus* in more northern parts of Switzerland will be possible in the future. Especially the region of Basel and Geneva seem to provide suitable conditions. This is supported by the fact that *A. albopictus* is further expanding in France along the Rhone valley and is already present in Grenoble [66]. Assumingly, the mosquito continuous its spread, both passively and actively, in more northern direction and will eventually be introduced to the Geneva region. In Germany, close to Basel, several traps have been found positive for *A. albopictus* [67, 68]. In view of these findings, the FOEN has commissioned the Swiss Tropical and Public Health Institute (Swiss TPH) and GLZ to launch a project on the surveillance of *A. albopictus* at the national level [69]. Ovitraps were set at resting areas along the South-North axis (A2, Chiasso-Basel), the West-East axis (A1, Geneva-St. Margreten), Valais (A9, Orbe VD-Brig) and Grisons (A13, St. Margreten-Bellinzona). In addition, other potential entry points like the national airports of Zurich, Geneva and Basel-Mulhouse, and the Rhine harbours in Basel were included in the surveillance (Fig. 1.7).

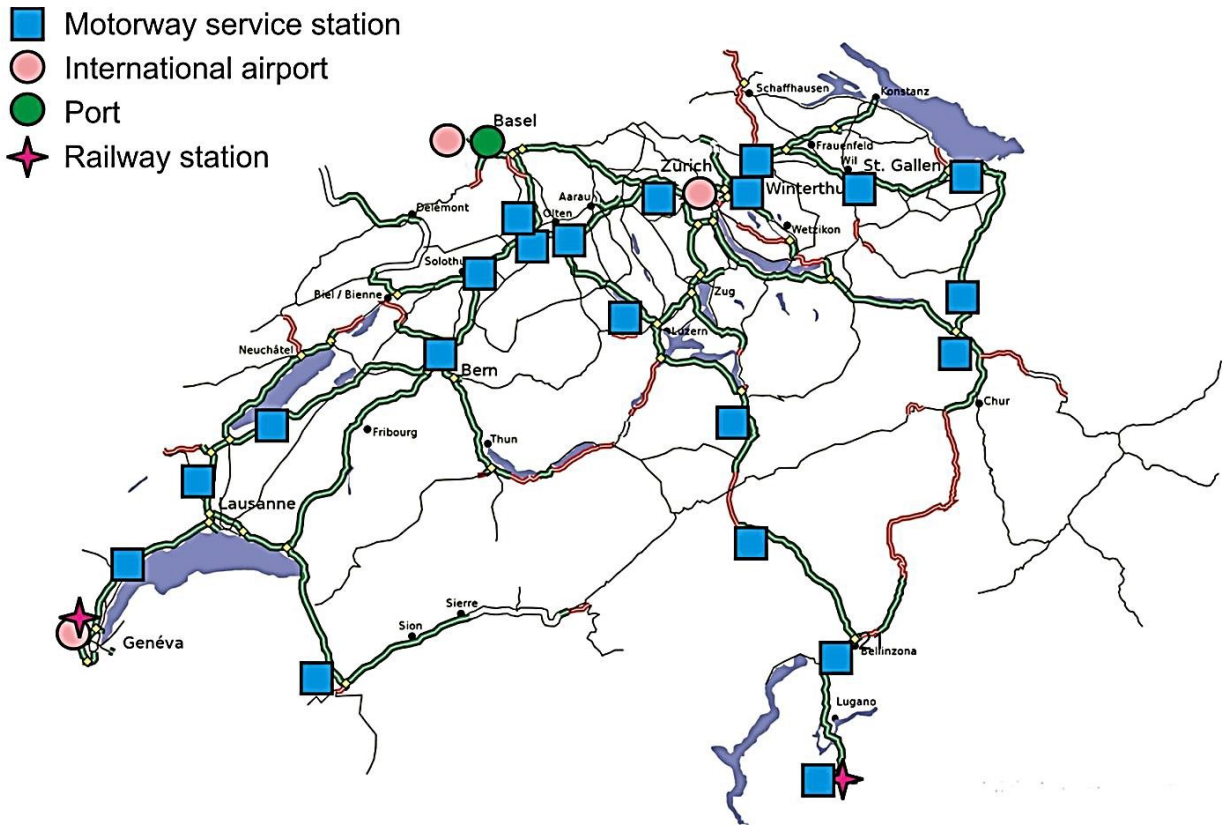

Figure 1.7: Locations of ovitraps in the frame of the national surveillance project on *A. albopictus* in Switzerland [69].

Figure 1.8: BG Sentinel trap (Biogents, Regensburg, Germany)

All ovitraps have been checked biweekly following the Ticino monitoring protocol. If ovitraps were found positive outside the Canton of Ticino, additionally traps for host-seeking mosquitoes were set. For this BG-Sentinel traps were used available from Biogents (www.biogents.com, Fig. 1.8). Traps were equipped with the BG lure for attracting host-seeking female *Aedes* mosquitoes.

In 2013, all traps within the Canton of Ticino have continuously been found positive. Three times, *A. albopictus* eggs have also been found at sampling sites located north of the alps, namely the motorway service stations Grauholz (A1, Canton of Berne), Gotthard Nord (A2, Canton of Uri), and Heidiland (A13, Canton of St. Gallen). However, these were isolated findings, indicating an introduction of few individual mosquitoes, most probably by road traffic. The national surveillance programme was repeated in 2014 and will be continued in 2015 and 2016.

1.5 Aims and objectives of this dissertation

The overall aim of the current PhD thesis is to study the biology of local *A. albopictus* population, assess the risk of disease transmission by *A. albopictus* by reviewing the effect of existing control measures in the canton of Ticino, and to assess alternative interventions in Switzerland.

As described in the previous section, the Swiss Canton of Ticino is undertaking large efforts for the surveillance and control of *A. albopictus* in its territory. Just across the border, in the neighbouring Italian Lombardy region, no intervention is in place. Since

the implementation of the control activities in the year 2003, its impact has never been evaluated. In this project, one aim is to investigate whether there difference in *A. albopictus* density in Italy as compared to Switzerland, assuming infestation being lower on the Swiss side of the border due to control activities. Therefore a randomised network of ovitraps, independent of the Ticino surveillance programme, was implemented, including the neighbouring Italian communities. The Ticino approach focuses on the surveillance of inhabited areas in order to fight the mosquito where it comes in contact with humans. It is therefore difficult to make inference on the spatial and temporal distribution of that mosquito species, even more so in non-urban sylvatic areas. The present approach aims at overcoming that gap by including sampling sites located in non-intervention and sylvatic habitats.

The second objective was to investigate the susceptibility of *A. albopictus* in Ticino to the range of insecticides that are currently applied as well as their alternatives. It is crucial for every control programme to confirm the target mosquito population is susceptible to the intervention in an area. Experimentally, a range of insecticide susceptibility bioassays for both larvae and adult *A. albopictus* mosquitoes were performed. The susceptibility status of *A. albopictus* from Switzerland was compared with the one from Italy, assuming the Italian population to be more susceptible because of not being exposed to any intervention. In addition, insecticide susceptibility was also compared to *A. albopictus* populations from Recife, Brazil, in order put the results in a broader context.

The third objective was to investigate host-feeding patterns of *A. albopictus* in the Swiss-Italian border region and in Recife. Adult female mosquitoes were caught with BG Sentinel traps and their blood meals analysed using PCR diagnostics. In terms of disease transmission scenarios it is important to know the host preferences of the vector to determine which viruses can be transmitted in an area and to assess the threat that a mosquito species poses to human health.

The fourth objective was to examine the vector competence of the Swiss *A. albopictus* population in transmitting DENV. It is well known that *A. albopictus* allows the proliferation and transmission of the virus. However, successful transmission of dengue viruses is temperature-dependent [70, 71]. Therefore infection experiments were performed under simulated Swiss climatic conditions using DENV serotypes 1, 2 and 3.

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Chapter 2

Surveillance and Control of Aedes albopictus in the Swiss-Italian Border Region: Differences in Egg Densities between Intervention and Non-intervention Areas

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RESEARCH ARTICLE

Surveillance and Control of *Aedes albopictus* in the Swiss-Italian Border Region: Differences in Egg Densities between Intervention and Non-intervention Areas

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Abstract

Background

Aedes albopictus, the Asian tiger mosquito, originates from the tropical and subtropical regions of Southeast Asia. Over the recent decades it has been passively spread across the globe, primarily through the used tyre trade and passive transportation along major traffic routes. *A. albopictus* is a proven vector for many arboviruses, most notably chikungunya and dengue, with recent outbreaks also in continental Europe. In southern Switzerland, in the Canton of Ticino *A. albopictus* was spotted for the first time in 2003. Since then the local authorities have implemented a control programme based on larval source reduction. Despite these efforts, mosquito densities have increased over the last decade, casting doubts on the effectiveness of such larval control programmes.

Methodology/Principal Findings

The Italian communities just across the Swiss-Italian border lack a control programme. This motivated us to compare the intervention and the non-intervention areas side by side in an attempt to find evidence for, or against, the effectiveness of larval *A. albopictus* control. Using ovitraps and a randomised sampling scheme, we examined the seasonal and spatial abundance of *A. albopictus* in sylvatic and urban environments across the Swiss-Italian border in 2012 and 2013. In the urban environments of the non-intervention area, egg densities were 2.26 times higher as compared to the intervention area. In the sylvatic environments, as compared to the urban environments, egg densities were 36% in the intervention area and 18% in the non-intervention area.

Competing Interests: The authors have declared that no competing interests exist.

Conclusions/Significance

Though alternative explanations are also valid, the results support the hypothesis that the Ticino intervention programme does have an impact. At the same time the data also suggest that current larval interventions fall short in gaining full control over the mosquito, calling for the evaluation of additional, or alternative, approaches. Ideally, these should also consider inclusion of the neighbouring Italian communities in the surveillance and control efforts.

Author Summary

The Asian tiger mosquito (*Aedes albopictus*) has gained increased attention in public health because it is a globally spreading, highly invasive mosquito species that may transmit several viruses. Outside of its original range in Southeast Asia it has been increasingly implicated in local transmission of chikungunya and dengue fever in many places including La Réunion, continental Europe, the Americas and Japan. The Asian tiger mosquito lays eggs that are adapted to desiccation and colder climate. This, together with the mosquito's ability to breed in almost any small, stagnant water body, makes its control extremely difficult, and there is much debate as to what interventions would be effective. This motivated us to compare the occurrence of the Asian tiger mosquito in southern Switzerland, where a mosquito surveillance and control programme is in place, with its neighbouring Italian districts where no such programme exists. The Swiss programme is based on public awareness campaigns to remove breeding sites and the use of insecticides against larvae. Using specialised traps that collect eggs from egg laying female mosquitoes, we found 2.26 times more *A. albopictus* eggs in the non-intervention area. The results support the hypothesis that targeting larval sources does have a significant impact.

Introduction

Aedes (Stegomyia) albopictus (Skuse, 1894), the Asian tiger mosquito, originates from the tropical and subtropical regions of Southeast Asia. During recent decades this mosquito species has spread to North America, Europe, Latin America and Africa, primarily by the transport of dormant eggs in used tyres [1] and through the importation of *Dracaena sanderiana* plants, also known as "lucky bamboo" [2]. At the regional level the mosquito is further passively dispersed through adults displaced by vehicles along traffic routes such as motorways [3].

Under laboratory conditions, *A. albopictus* is a competent vector for at least 26 arboviruses, notably chikungunya, dengue, yellow and West Nile fever [4,5]. *A. albopictus* is also of veterinary significance because it is equally a competent vector for *Dirofilaria immitis*, a nematode that causes dirofilariosis in dogs [4]. Therefore, the establishment of *A. albopictus* represents a potential threat for both public and veterinary health. How realistic this threat is also for mainland Europe has been clearly demonstrated by several reports of autochthonous chikungunya and dengue cases over the recent years. In 2007, an outbreak of chikungunya associated with the establishment of *A. albopictus* occurred in Ravenna, Italy, with over 200 confirmed cases [6,7]. More recently, between August and September 2010, autochthonous cases of dengue have been reported from Croatia and metropolitan France with *A. albopictus* deemed responsible for its transmission [8,9]. In the same year, two people became also infected with the

chikungunya virus in Fréjus, France [10]. Then additional autochthonous dengue cases were reported from southern France in 2013 [11] and again in 2014, alongside new cases of chikungunya [12].

In Italy, *A. albopictus* was first detected in Genoa in 1990 from where it spread to many parts of Italy, including the border region south of Switzerland [13]. In response to its presence in northern Italy an *A. albopictus* surveillance programme was put in place by the local authorities in southern Switzerland in the Canton of Ticino (in the following simply called Ticino) in 2000. Three years later, the first *A. albopictus* eggs were detected [14]. As increasing egg numbers were detected between 2003 and 2006, the surveillance effort was gradually intensified and control measures implemented [14]. Control measures entailed removing of potential breeding sites and use of larvi- and adulticides. In the following years the estimated *A. albopictus* density was still low, suggesting that individual adult mosquitoes had been sporadically introduced from Italy but had not yet established a sustained population in Ticino. Yet, in 2007 the situation changed significantly, when a dramatic increase of positive mosquito traps in Chiasso, right at the Swiss-Italian border, was observed, indicating that a local mosquito population had then been established [14,15].

In 2007 the monitoring system consisted of 292 oviposition traps (ovitraps) that were regularly controlled, covering a defined area of approx. 4.6 km^2. Ovitraps are a widely used tool for the surveillance of container breeding *Aedes* [14,16–19] as they are sensitive, relatively inexpensive and easy to maintain [20][16,18]. The ovitrap is a device that consists of a water-filled black bucket with a piece of wood, or styrofoam, onto which female mosquitoes may deposit their eggs. In Ticino, the ovitraps used consist of a flower pot filled with water into which a wooden strip is plunged for the females to lay eggs [14]. The traps are set within communities as well as at lay-bys and service areas along the motorway E35 [21]. The E35 is a south-north European route that runs from Rome (Italy) to Amsterdam (the Netherlands). In addition, places with stagnant water that cannot be averted otherwise were treated with *Bacillus thuringiensis* var. *israelensis (Bti)*, a biological control agent for larval mosquito stages [22].

During the last years, the ovitrap network has been continuously expanded and adapted. Today, over 1,000 ovitraps are deployed within the frame of the Ticino surveillance and control programme covering an area of approx. 60 km^2. The traps are inspected biweekly and the number of positive traps serves as an indicator if and where the application of insecticide would be necessary [14]. In addition, information campaigns are carried out to raise public awareness in order to sensitise residents for the occurrence of *A. albopictus* and to eliminate potential breeding sites from their private properties. Despite these measures *A. albopictus* densities have still increased in Ticino over the last decade [14].

Larval source reduction by removing water containers that may serve as breeding sites is considered the best method for the control of *A. albopictus* by several authors [23,24]. Studies from North Carolina [25], Spain [26] and New Jersey [27] reported that source reduction campaigns resulted in a temporary suppression of immature *A. albopictus*. Indeed, Bartlett-Healy et al. [28] showed that artificial containers on private properties are the most productive sources for the emergence of *A. albopictus*, highlighting the importance of public involvement in the overall control effort. Awareness campaigns showing the public how to identify and eliminate potential breeding sites from their properties have become an integral component of *Aedes* mosquito control [20]. Such campaigns go often hand in hand with larvicide treatments and spraying of insecticides targeting adult mosquitoes. Comparing different intervention approaches, Fonseca et al. [27] concluded that careful source reduction by trained personnel, in combination with efforts to educate the public in removing breeding sites, results in a significant decrease in adult *A. albopictus* numbers.

Despite the above evidence there is still much debate as to how effective such larval control measures really are, particularly in areas where mosquitoes are continuously re-introduced such as being the case in southern Switzerland. This motivated us to examine the potential impact of the current surveillance and control programme by comparing relative mosquito densities between Ticino and two neighbouring Italian provinces where ecological parameters are comparable; yet, no intervention programme is in place.

Methods

Study area

Field surveys were carried out from July to November 2012 and from May to November 2013. The study area enclosed the southernmost border region of Ticino, the Mendrisiotto district, and the provinces of Varese and Como in Lombardy, Italy (Fig 1). Hereafter, the part of the study area in Ticino is called the "intervention" area and that of Varese and Como the "non-intervention" area. In total, the study area covered a surface area of 118 km^2; 65 km^2 on the Italian side and 53 km^2 on the Swiss side of the border. The difference in the surface areas were to make up for places that were either inaccessible or covered by the Lake of Como.

The landscape of the study area is similar on both sides of the border and dominated by deciduous forests and agriculture. Approximately 20% are covered by buildings or roads. Population densities are almost equal and are 440 and 480 inhabitants per km^2 in the Ticino and the Lombardy part, respectively [29,30].

The traffic-intense European route E35 runs through the study area, connecting the South of the continent with North-western Europe. On average, on a single work day over 62,000

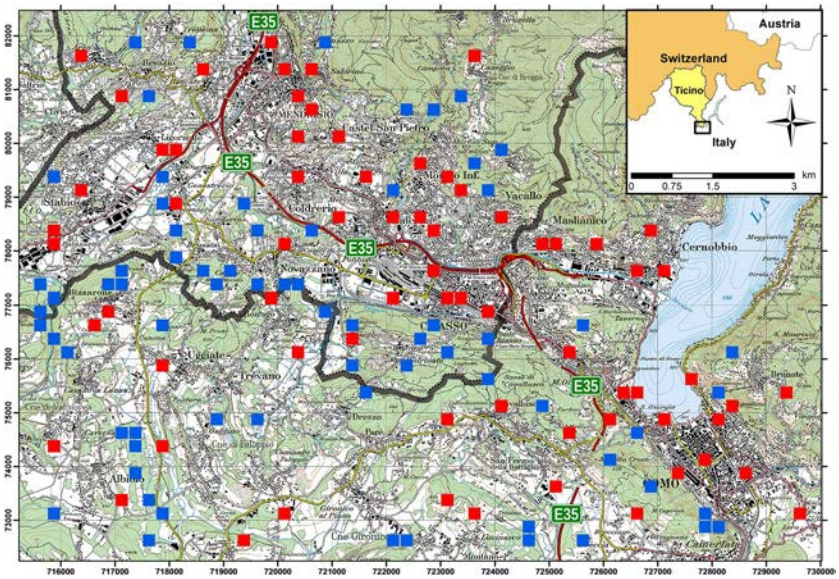

Fig 1. Study area and ovitrap positions. The red and blue squares represent sampling grid cells in urban (red) and sylvatic (blue) environments. In each country 35 grid cells were randomly allocated to either the urban or sylvatic environment. Within selected grid cells two ovitraps were placed at a minimum distance of 50 m between them to avoid interference in mosquito attraction. In total, there were 280 ovitraps (2 countries x 2 environments x 35 cells x 2 ovitraps). The thick grey line denotes the Swiss-Italian border with the intervention area (Ticino, Switzerland) in the North and the non-intervention area (Lombardy, Italy) in the South. The orange line, crossing the Swiss-Italian border, shows the European route E35. The numbers at the left and at the bottom indicate the Swiss km co-ordinates.

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people cross the Swiss-Italian border, mostly by car [31,32]. Most of the people crossing the border commute to Switzerland for work.

The climate in the study area is continental with relatively mild temperatures, yet distinct annual seasons. Mean annual temperature and rainfall are 11.1˚C and 1,311 mm [33]. Besides its relatively sunny weather, the region is also well known for its heavy thunderstorms during the summer.

Using the ArcGIS version 10.0 (ESRI Inc., USA) geographic information system (GIS) software a grid with 250 m by 250 m cells was virtually superimposed over the study area. From this grid, all grid cells within a lake and those that were inaccessible in the field were excluded from sampling. The remaining grid cells were then stratified into "urban" and "sylvatic" environments. A cell was classified as sylvatic if at least 50% of the surface were covered with trees, and vice versa. For each of the four combinations of area and environment 35 cells were randomly picked from the grid to avoid sampling bias. For this purpose the cells were first numbered through and then the numbers drawn using a random number generator. The total number of cells included in the study was chosen on the basis of a power calculation that used simulation methods described in Johnson et al. [34]. For this exercise we assumed a minimal effect size of 10% difference in egg counts between the two countries and a power of $1-\beta = 0.8$.

Ovitrap sampling and species identification

Relative densities of *A. albopictus* were estimated using ovitraps. The traps mimic breeding sites, attracting gravid females to deposit their eggs. In the present study, an ovitrap consisted of a 1.5 l, black plastic flower pot, filled with 1.2 l tap water. Three small holes with a diameter of 5 mm were drilled at equal distances, 2 cm below the rim, to prevent the trap from being flooded by rain. A wooden strip made of untreated beech wood was placed inside the pot so that it was partially submerged and partially sticking out of the water. The strip measured 20 cm x 2.5 cm x 0.5 cm. In order to prevent the ovitraps from becoming potential breeding sites larvicide granules of *Bti* (VectoBac, Valent BioSciences, USA) were added. The strips, water and *Bti* were replaced biweekly. When replaced, the traps were cleaned and the wooden strips wrapped in clingfilm for transportation and preservation. Each strip was labelled with the date and a unique code together with additional information related to the trap condition and the presence of larvae. The final trap position within the assigned sampling grid cell was chosen in the field. Traps were placed at shaded, wind protected locations that, in the optimal case, were surrounded by green vegetation as done in previous studies (e.g. [14,27]). All traps were geo-referenced with a handheld GPS device (nüvi 1390, Garmin, Switzerland).

In the laboratory, the strips were inspected for the presence of mosquito eggs using a stereo microscope (EZ4D, Leica Microsystems, Germany) and, where present, the number of eggs counted. During the first season in 2012, eggs were identified to species level by morphology. At that time only two container-breeding mosquito species, *A. albopictus* and *A. geniculatus*, were known to occur in the region. Both species can easily be distinguished by morphology [20,35]. As a quality control measure an additional identification method was introduced for the 2013 mosquito season. Here, for each collection round, eggs from two randomly selected positive traps were also analysed by matrix-assisted laser desorption/ionization mass-spectrometry (MALDI TOF MS) [35]. Only eggs were chosen for the analysis that had previously been morphologically determined as being *A. albopictus* and, where present, were still intact. For MALDI-TOF MS three to five apparently intact eggs were carefully removed using forceps from the ovitrap strips and then transferred to 1.5 ml Eppendorf tubes. The samples were sent to Mabritec SA (Riehen, Switzerland) where they were prepared and analysed according to the protocol described in Schaffner et al. [35].

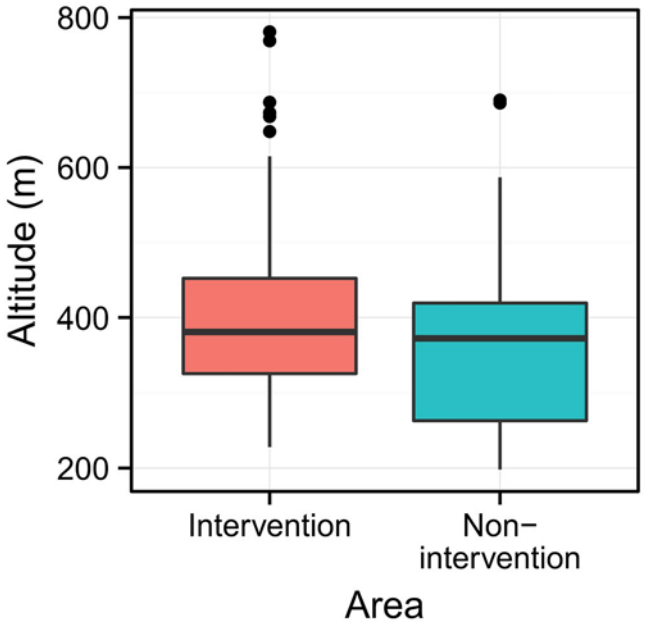

Fig 2. Altitude range of trap positions. The boxplots show the distribution of the altitude above sea level for the 140 ovitraps in each of the two areas. The boxes represent the interquartile distances (IQD), while the centrelines through each box show the medians. The dots indicate outliers and the whiskers extend to the extreme values of the data, calculated as ±1.5 x IQD from the median.

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Data analysis

The numbers of *A. albopictus* eggs on each wooden strip were counted and recorded in an Excel data base together with additional information such as the trap location, date, condition of the trap, etc. Data were then imported into the GIS software ArcGIS Version 10.1 (ESRI Inc., USA) to produce spatio-temporal density maps. For statistical analysis, data were loaded into the freely available software R, version 3.1.2 [36].

Relative egg densities per trap were modelled by a zero-inflated negative binomial (ZINB) regression model using the R package "glmmADMB" [37,38]. The ZINB accounted for an excessive number of zeros in the ovitrap count data. In the ZINB model, the outcome was the biweekly egg count per trap, while the predictors "area" (non-intervention vs. intervention) and "environment" (urban vs. sylvatic) and their interaction were included as fixed effect terms. To account for the slight bias in altitude towards higher elevations in the intervention area (Fig 2) and the potential relationship between altitude and temperature, a predictive term for "altitude" was also included in the model. Altitude was entered as metres above sea level. As egg counts were repeatedly (i.e. biweekly) measured for the same ovitrap, an intercept was included for "trap" as a random term in the ZINB model, accounting for correlations in the number of eggs caught in the same trap. Also included as a random term was an intercept for the week in which the traps were replaced in order to account for seasonal variations. The model was also inspected for signs of spatial correlations in the residuals using the variogram function in the R package "gstat", version 1.0–19 [39]. The statistical graphics were produced with ggplot2, version 1.0.0 [40]. The level of significance was set at $\alpha = 0.05$.

Results

In 2012, ovitrap collections ran over 20 weeks (i.e. 10 rounds) from July to November, while the survey covered 26 weeks (i.e. 13 rounds) from May to November in 2013. The first eggs in

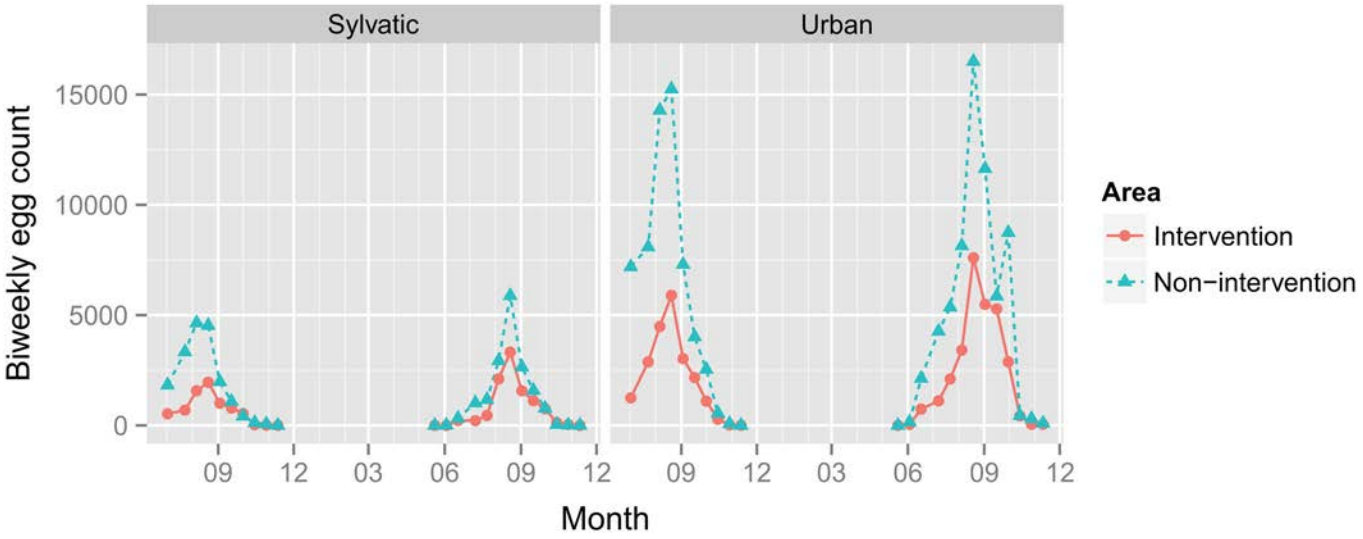

Fig 3. Temporal distribution of *Aedes albopictus* in the Swiss-Italian border region. The numbers of *A. albopictus* eggs found in the ovitraps are shown as sums over all 70 traps for each combination of environment and area. In the calendar week 38 in 2013, an unusually high number of ovitraps was dysfunctional (e.g. traps were found turned over, damaged or missing; S1 Table), explaining the sudden drop in the curve for the non-intervention area in the urban environment.

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the season were found in early June, followed by a steady increase with a peak between 19 and 26 August. In September, egg counts dropped again and eventually ceased in mid-November (Fig 3).

From the potentially 6,440 available strips for the analysis (280 traps x 23 rounds), 357 (5.5%) have gone missing (Table 1 and S1 Table); either they have been taken from the traps or the traps themselves became dysfunctional (e.g. traps were found turned over or missing completely). From the remaining 6,083 strips, 2,508 (41.5%) were positive for *A. albopictus*, 689 (11.4%) for *A. geniculatus* and 333 (5.5%) for both species. While for *A. albopictus* a total of 224,728 eggs were counted, egg numbers were not recorded for *A. geniculatus*, only whether eggs were present or absent.

In 2012, egg counts per trap ranged from 0 to 1,537 in the non-intervention area (i.e. Lombardy, Italy) and from 0 to 441 in the intervention area (i.e. Ticino, Switzerland). In 2013, egg counts ranged in the non-intervention and intervention area from 0 to 1,039 and from 0 to 1,333, respectively. Egg counts were generally higher in the non-intervention area (Table 1). In all (i.e. 20) instances the morphological identification was confirmed by MALDI-TOF MS.

Remarkably, *A. albopictus* eggs were found across the whole altitude range (Fig 2) and were even repeatedly found at higher altitudes up to 781 m above sea level (S1 Table).

Table 1. Summary of the biweekly *Aedes albopictus* egg counts.

Area	Year	Strips analysed	Strips missing	Positive strips	Egg count per strip					
					Minimum	1st quartile	Median	Mean	3rd quartile	Maximum
Intervention	2012	1,370	30	563 (44.1%)	0	0	0	20.6	16	441
	2013	1,677	143	550 (32.8%)	0	0	0	23.3	13	1,333
Non-intervention	2012	1,375	25	707 (51.4%)	0	0	1	56.2	51.5	1,537
	2013	1,661	159	688 (41.4%)	0	0	0	48.2	41	1,039

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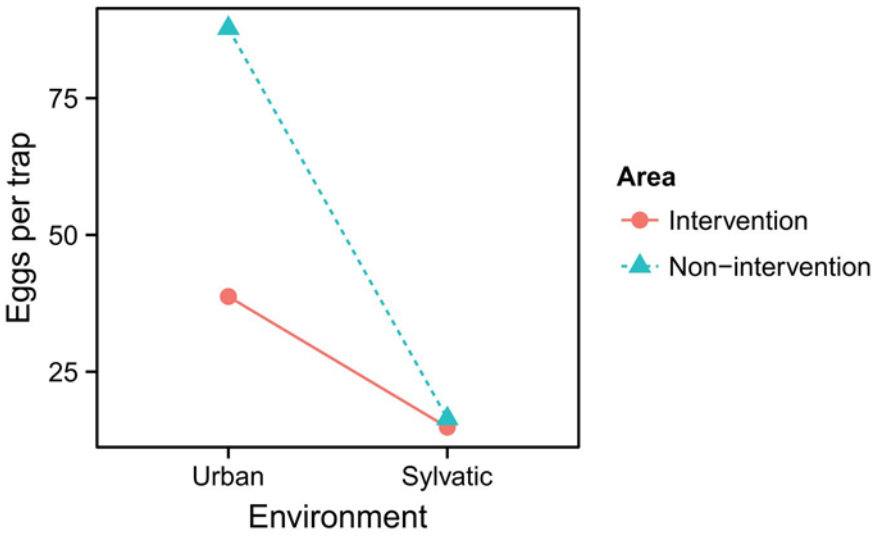

Fig 4. Effects of "area" and "environment" on average egg counts. The difference in average egg counts between the urban and sylvatic environments in the intervention area was half the difference between the environments in the non-intervention area. Note that the average egg numbers represent the mode from the back-transformed coefficients.

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In the urban environment, the average ratio in egg densities between the non-intervention and the intervention area was 2.26 (95% confidence interval, CI: 1.40–3.65; Fig 4 and Table 2). Mosquito eggs were also detected in the sylvatic environment, although, as compared to the urban environment, the counts were much lower. The average ratios between the sylvatic and the urban environments were 0.36 and 0.18 in the intervention and in the non-intervention area, respectively. In the model, the difference in these ratios is accounted for by the interaction term (Table 2) with an estimated ratio of 0.504 (CI: 0.254–0.997) and graphically illustrated in Fig 4. In addition, the model improved by adding a term for altitude; an increase of altitude by one meter decreases egg counts by a ratio of 0.995, that is by 0.54% (95% CI: 0.37%– 0.71%). The model did, however, not improve when adding "year" as a term, indicating that egg counts did not significantly differ between the two years ($\chi^2 = -2.6$, $p = 1$). Moreover, inspecting the residuals for spatial correlations did not detect violation of independence.

When plotting the positive traps on the geographic map, it becomes apparent that not only the numbers of eggs were higher in the non-intervention area but, equally, more traps were positive (Fig 5). The picture remained the same in both years and in the early (July) and late

Table 2. Result summary for the zero-inflated negative binomial model (ZINB). The ZINB predicts the average number of eggs caught in an ovitrap as a function of the predictors.

Predictor	Coefficient β ($\log_2$)	SE(β) ($\log_2$)	z-value	p-value
Intercept	3.675	0.729	5.04	< 0.0001
Area (non-intervention)	0.817	0.244	3.35	< 0.001
Environment (sylvatic)	-1.021	0.253	-4.04	< 0.0001
Interaction: Area (non-intervention) x Environment (sylvatic)	-0.686	0.348	-1.97	< 0.05
Altitude	-0.005	0.001	-6.13	< 0.0001

Negative binomial dispersion parameter: 0.651 (SE = 0.035). Zero-inflation: 0.315 (SE = 0.014). The variances of the random intercepts for "trap" and "week" were 1.904 (SD = 1.38) and 9.046 (SD = 3.008), respectively. Number of observations: total = 6,083; trap = 280; week = 23.

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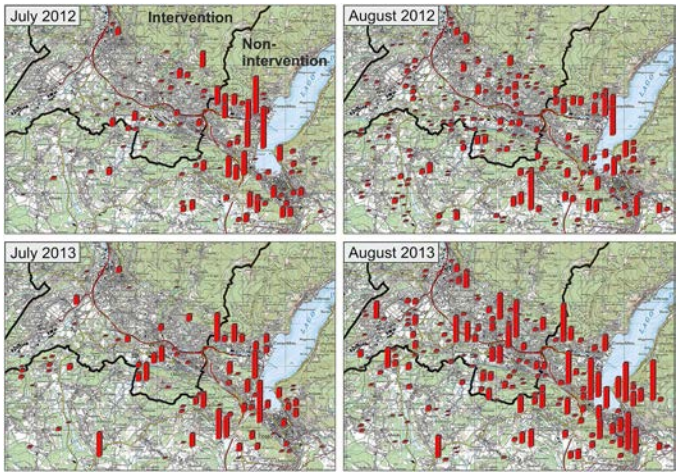

Fig 5. 2012 and 2013 early and peak season trapping data. The size of the red bars represents the number of eggs found in the ovitrap (the smallest bars represent 1 to 50 eggs, the largest 900–1,500). The thick black line marks the Swiss-Italian border; the red line represents the European motorway E35. To enable visibility of all bars, some are slightly shifted to the right.

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(September) mosquito season. Combining egg counts from both seasons, 32.4% (72,869 eggs) of the *A. albopictus* eggs were collected alone in the city of Como. In Switzerland the communities of Chiasso and Balerna, which are located at (i.e. Chiasso) or very close to (i.e. Balerna) the border, had the highest *A. albopictus* egg counts. Over both sampling periods, total egg numbers in Chiasso and Balerna were 12,637 and 12,212, respectively. Together they represent 11% of the total *A. albopictus* egg count. As traps were, however, distributed randomly to make inference about the whole region these numbers have to be interpreted with caution.

Discussion

Our results show that in the urban environment the *A. albopictus* egg density was 2.26 times higher in the non-intervention area, on the Italian side of the border, as compared to the intervention area in Ticino. We also found that the ratio in egg densities between the urban and sylvatic environment was twice as high in the non-intervention area. Together, although not yet fully conclusive, the results are in line with the hypothesis that the Ticino control strategy of larval source reduction does affect *A. albopictus* in the urban environment.

In Ticino, the backbone of the *A. albopictus* control programme consists of larval source reduction through public awareness campaigns and larviciding [14]. Public awareness campaigns use multiple communication channels, including the media, internet and leaflets. As a result, artificial containers such as flower pots or water storage tanks are routinely turned over or covered. Larviciding consists of monthly applications of diflubenzuron or weekly treatments with *Bti* in the public space during the main mosquito season from May to October. Citizens are also encouraged to treat water bodies in their gardens that may not be avoided otherwise with commercially available *Bti* pellets. Certain areas such as school yards, or areas from where imported cases of chikungunya or dengue have been reported, are also sprayed with permethrin targeting adult mosquitoes [14]. In contrast to the coordinated efforts in Ticino, we are not aware of such a control programme in the Italian communities close to the Swiss-Italian border. We, therefore, hypothesise the observed differences in egg densities being attributable to the bias in mosquito control efforts. Preliminary results (S1 File) as well as the personal experience from the field made by the authors do suggest more breeding sites being present in the

Italian communities. It would have been desirable to systematically quantify the presence and characteristics of breeding sites, and include in the analysis the actual amount of insecticides applied in both the intervention and non-intervention areas. Unfortunately, our resources were limited; and hence including such data was beyond the scope of the current study. However, it has to be noted that even by having that data available we would still not be able to reach a conclusive answer as the observations might still be correlated to yet another unknown variable. A much more powerful approach would be a trial in which the impact is measured in response to the implemented intervention.

Despite the above limitations, the results are in line with the few previous studies that have investigated the effects of larvi- and adulticiding [23–27]. It is also recognised that the positive effect of interventions in public areas may be strongly boosted by involving the general public in removing potential breeding sites from their own properties. Correspondingly, Vanlerberghe et al. [41] found that by engaging the public in reducing larval breeding sites in a routine vector control programme can reduce *Aedes* infestations by 50–75%. The other positive effect is this concept ensures better embedding of mosquito control in the social, cultural, political and economic context [42].

In the present study we used egg counts from ovitraps to estimate and compare *A. albopictus* densities because these traps are sensitive at low mosquito densities [43], are cheap and run independently of electricity or a source of carbon dioxide. There are, however, concerns over the validity of using ovitraps for density estimates because a single female may place its eggs in multiple sites [37], or the ovitraps may compete with nearby breeding sites (see e.g. [44]). Intriguingly, Carrieri et al. [45] found that ovitrap data were a reliable alternative for the mean number of biting females per unit area as well as larval productivity. Similarly, Facchinelli et al. [46] found a good correlation between sticky trap catches of adults and egg counts in ovitraps. Perhaps some studies might have failed in finding a relationship between egg counts and other sampling methods due to the use of derived statistics from non-normally distributed egg counts rather than working directly from the actual counts as done here.

In the present study, ovitraps even up to 781 m altitude were found repeatedly positive for *A. albopictus* eggs throughout the entire season. It has previously been assumed that eggs are unlikely to survive winter conditions at such altitudes even in warmer climatic conditions [47]. Although we cannot fully exclude rapid re-colonisation in spring or repeated re-introductions during summer, our observations suggest local reproduction rather than sporadic introductions. Altitude was also included as a covariate in the statistical model to account for the heterogeneity in elevation, and to some extent also temperature, across the entire study area.

In its native range *A. albopictus* is a tree hole-breeding mosquito, yet it is perfectly adapted to the man-made urban environment [48], where blood sources and (artificial) breeding sites are more readily available, demonstrated here by the much higher mosquito densities in the urban environments. As a consequence, focusing control efforts in urban areas is expected to be more effective though forests may still serve as reservoirs. Implementing control measures in forested areas is, however, even more challenging if not impossible due to the ban of using insecticides in forests [49].

Intriguingly, most ovitraps in Switzerland were still negative earlier in the season, when in Italy many traps had already been positive for *A. albopictus*. How can we explain this pattern? One explanation would be that the early season intervention in Ticino successfully eliminates the first mosquito generation in the year, resulting in lower reproduction. Also treatments that have been done by the end of the previous season could contribute to the observed pattern. A third explanation would be that we observe a boundary effect due to e.g. climatic constraints [50]. In the latter scenario, mosquitoes are annually re-introduced from Italy, rather than being stable overwintering populations, so that in Ticino numbers manage to pick up only later in

the season. This raises the question as to what extent the Ticino *A. albopictus* population has firmly established in Switzerland. In other words, how many egg batches from the previous year have actually survived the winter? A study on the population genetic structure might shed light on the above question. Besides this being a question of academic interest, knowing how mosquitoes propagate and leak into the control area would also help in improving intervention strategies. In this context, Talbalaghi [51] found in the Italian region Piedmont that, without concerted actions between neighbouring municipalities, the long term effect of the control efforts were undermined. Therefore, we would strongly advocate the development and implementation of a transnational action plan for the surveillance and control of *A. albopictus* in the Swiss-Italian border region. Given how local residents mostly welcomed us to set the traps on their private properties and their keen interest in our work, we are very positive that an interregional action plan would receive a lot of support from the public.

Conclusions

We found that *A. albopictus* egg densities in the non-intervention area on the Italian side of the Swiss-Italian border were more than twice compared to the intervention area in Ticino. Though other factors might explain the difference in mosquito densities, the present data support the hypothesis that the currently implemented surveillance and control programme in Ticino has a positive impact. Presumably public awareness is a major component in reducing *A. albopictus* densities. However, it remains to be shown experimentally how big the actual impact of the current interventions really is.

Supporting Information

S1 Table. Original data set with egg counts for each wooden strip. Each line corresponds to a single strip/observation. TRAP.ID = unique identifier for each trap location; WGS84.LAT and WGS84.LNG = geographical coordinates (i.e. latitude and longitude) in the World Geodetic System format WGS84; ALTITUDE = metres above sea level; AREA = area, intervention (Ticino, Switzerland) and non-intervention (Lombardy, Italy); MUNICIPALITY = municipality, the administrative division; ENVIRONMENT = "sylvatic" or "urban" environment; DATE = day when strip was removed from the trap in the field; N.ALBOPICTUS = number of *Aedes albopictus* eggs on the strip ("NA" means the strip was missing); GENICULATUS = logic variable indicating the presence of *Aedes giniculatus* eggs on the strip.
(XLSX)

S1 File. Characterisation of potential breeding sites from 8 randomly selected sampling grid cells.
(DOCX)

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Author Contributions

Conceived and designed the experiments: TTS PM MAVdMS. Performed the experiments: TTS BFF. Analyzed the data: PM TTS. Contributed reagents/materials/analysis tools: PM TTS EF LE MT. Wrote the paper: TTS EF LE MT LNR MAVdMS PM.

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Chapter 3

First report of the invasive mosquito species *Aedes koreicus* in the Swiss-Italian border region

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Parasites & Vectors

SHORT REPORT Open Access

First report of the invasive mosquito species *Aedes koreicus* in the Swiss-Italian border region

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Abstract

Background: In 2012 and 2013, an entomological survey of *Aedes albopictus*, the Asian tiger mosquito, was carried out in the border region of southern Switzerland and northern Italy, using ovitraps. In July 2013, besides *A. albopictus* already known to the region several unusual eggs were recovered.

Findings: A total of 548 seemingly different eggs were found within three communities: Chiasso (Switzerland), and Como and Brunate (Italy). Proteomic diagnostics based on matrix-assisted laser desorption/ionization mass-spectrometry (MALDI-TOF MS) and morphological identification of one reared adult revealed the presence of at least 18 *A. (Finlaya) koreicus* (Edwards, 1917) specimens. *A. koreicus* is a species native to Southeast Asia and is competent to transmit Japanese encephalitis and potentially other arboviruses, as well as the dog heartworm *Dirofilaria immitis*. While new to Switzerland, this invasive species has previously been reported from Belgium, north-eastern Italy and European Russia.

Conclusions: This is the first report of the introduction of this exotic mosquito species into Switzerland and Lombardy, Italy, suggesting the range of *A. koreicus* is expanding in Central Europe. As *A. koreicus* is competent to vector pathogens its establishment imposes a risk to public and veterinary health. From a technical point of view, the presence of *A. koreicus* alongside *A. albopictus* requires careful analysis and reliable diagnostics. As a diagnostic tool the use of the recently developed MALDI-TOF MS approach has proofed to be a very useful approach, particularly since hatching rates of *A. koreicus* seem to be low, making identification by classic morphology difficult, if not impossible.

Keywords: Invasive species, Mosquito surveillance, Mosquito diagnostics, MALDI-TOF MS

Background

After the introduction and establishment of the Asian tiger mosquito, *Aedes albopictus* (Skuse), in the Swiss Canton of Ticino in 2003, an entomological surveillance and control programme was instigated by the canton's mosquito working group, Gruppo cantonale di Lavoro Zanzare (GLZ) [1]. GLZ uses ovitraps for the surveillance of *A. albopictus* as a basis for interventions in urbanised areas where the mosquito has become endemic [2]. Intervention is chiefly based on larval source reduction through public awareness campaigns that include the distribution of leaflets, a hotline and a website, and by larviciding using diflubenzuron and *Bacillus thuringiensis* var. *israeliensis (Bti)*, targeting small water containers (i.e. < 200 L) such as catch basins, plant saucers, drums, buckets, tarpaulins, tyres and bathtubs [2]. Larger water bodies were inspected for the presence of larvae using standard dippers (model 1132, BioQuip Products, Rancho Dominguez, USA). If immatures were present, the breeding sites were also included in the control approach.

In 2012 and 2013, as part of a research project the mosquito survey was expanded across the Swiss-Italian border, including the Mendrisiotto district (Switzerland) and the northern part of the Lombardy (Italy). In total, the study area covered a surface area of 118 km^2, 65 km^2 on the Italian and 53 km^2 on the Swiss side of the border (Fig. 1). Using the ArcGIS version 10.0 (ESRI Inc., USA) geographic information system software a grid with cells measuring 250 m by 250 m was superimposed over the study area. Seventy grid cells were then randomly selected in both countries using the "sample()"

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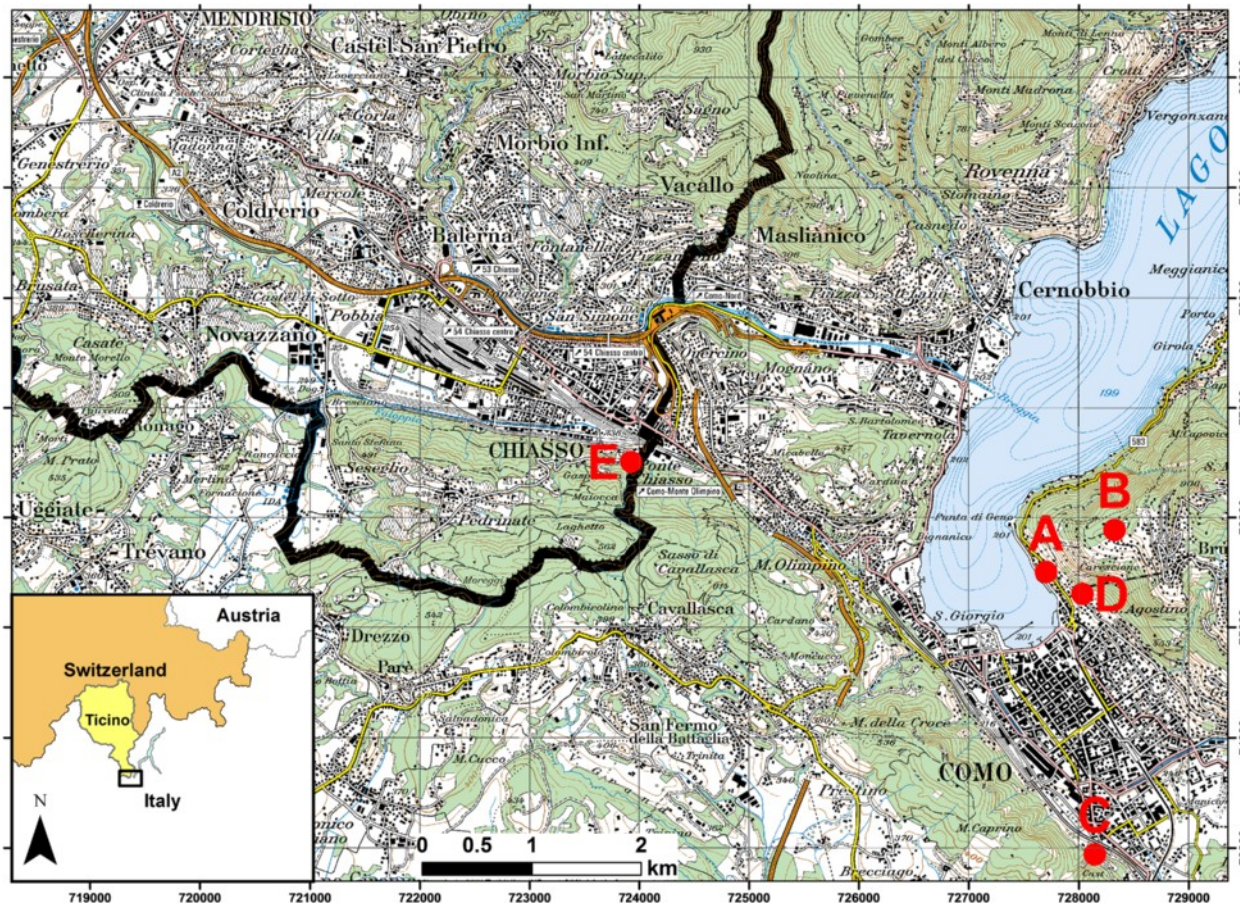

Fig. 1 Locations of ovitraps positive for *Aedes koreicus* across the Swiss-Italian border in 2013. Details for the locations and identified eggs are given in Table 1. The numbers at the border indicate the Swiss grid km coordinates. The thick black line represents the Swiss-Italian border. Map layers were purchased from the Swiss Federal Office of Topography

function in the statistical software R version 2.11 [3]. Within each selected grid cell, relative *A. albopictus* densities were measured by placing two ovitraps, set apart at a distance of at least 50 m to avoid interference in attraction. All traps were geo-referenced with a nüvi 1390 (Garmin, Switzerland). Field surveys were carried out from July to November in 2012 and May to November in 2013.

Ovitraps comprised 1.5 L, black plastic flower pots (Ramona Hydro, Luwasa, Switzerland) filled with 1.2 L tap water. Three equally spaced holes were drilled 2 cm below the rim to prevent the traps from being flooded by rain. A wooden slat made of untreated beech wood, measuring 20 cm × 2.5 cm × 0.5 cm, was placed inside the pot so that it was partially submerged and partially stuck out of the water. To prevent the ovitraps from becoming potential larval habitats, larvicide granules of *Bti* (VectoBac®, Valent BioSciences, USA) were added. The slats, water and *Bti* were replaced biweekly. The slats were individually labeled and wrapped in cling film for transport and preservation.

Findings

By the end of July 2013, a total of 19,328 mosquito eggs from 280 ovitraps were collected across the study area. For five traps (Table 1 and Fig. 1) placed within the communities of Chiasso, (Switzerland), Como and Brunate (Italy), routine egg inspections in the laboratory revealed unusual eggs that were very similar to *A. albopictus* and yet appeared different to the trained eye. Though morphologically not entirely distinct, the eggs seemed to be somewhat more elongated and pointed. In total, we noticed 548 such unusual eggs.

In the laboratory, the wooden slats collected from the ovitraps in Switzerland were incubated in tap water in an attempt to hatch the eggs. The water was allowed to settle for 24 h before use. The eggs were incubated for 7 days in a climate-controlled chamber (KBWF 720 E5.2, Binder GmbH, Germany) at a temperature of 28 °C, a relative humidity of 70 % and a light:dark cycle of 16:8 h. Unfortunately, only one larva hatched from which a female imago emerged. The specimen was morphologically identified as *A. (Finlaya) koreicus* (Edwards, 1917) using the taxonomic

Table 1 Field-caught mosquito eggs identified as *Aedes koreicus* across the Swiss-Italian border in 2013

Trap[1]	Position	Community	Total egg number	Suspected as *A. koreicus*	Confirmed as *A. koreicus*
A	45.819 N, 9.082E	Brunate (Italy)	249	131	3
B	45.822 N, 9.090E		47	21	2
C	45.796 N, 9.087E	Como (Italy)	273	125	3
D	45.817 N, 9.086E		402	201	7
E	45.829 N, 9.033E	Chiasso (Switzerland)	92	70	3[2]

[1]The letters correspond with Figure 1

[2]All eggs from trap E remaining after the MALDI-TOF MS analysis were incubated in tap water. One female imago emerged

key of Ree [4], and later confirmed by Francis Schaffner (pers. comm.), a renowned expert in mosquito taxonomy. The identified female corresponded to the morphological form known from the South Korean volcanic island Jeju-do [5]. This form has also recently been reported from Belgium [6] and north-eastern Italy [7].

Given the low hatching rate, a subsample of two to six eggs per slat (Table 1) were tested by matrix-assisted laser desorption/ionization mass-spectrometry (MALDI-TOF MS) in combination with a validated database, curated at Mabritec SA (Riehen, Switzerland) [8, 9]. Eggs selected for analysis were deposited close to each other and at a maximum distance from the eggs suspected to be *A. albopictus*. For the MALDI-TOF MS analysis the eggs were carefully detached from the slat with a brush and then prepared and processed as described by Schaffner et al. [9]. All selected egg specimens were determined as *A. koreicus* (n = 17, Table 1).

Discussion and conclusions

A. koreicus was originally found in Korea, Japan, China and Eastern Russia [5]. Its introduction has previously been reported from Belgium in 2008 [6], north-eastern Italy in 2011 [7] and European Russia in 2013 [10]. Meanwhile, the mosquito species has successfully established local populations in Belgium and north-eastern Italy [6, 11], confirming its ability to colonise new areas in temperate regions. Although in nature *A. koreicus* breeds in rock pools and tree holes, it successfully utilises artificial breeding sites in more urban environments, very much like other invasive *Aedes* species, including *A. albopictus* and *A. japonicus* (Theobald). Host seeking females may feed on humans and domestic animals both during the day and at night [12]. At the end of the annual season when daylight becomes shorter, *A. koreicus*, like other members of the *Aedes* group, deposits eggs that are dormant and more resistant to desiccation and cold temperatures than eggs laid during the season. Dormant eggs catalyse the mosquito's passive distribution to new areas [13]. Capelli et al. [7] showed that *A. koreicus* dormant egg stages are even more resistant to cold temperatures compared to *A. albopictus*; and hence this species has the potential to colonise a much wider area of Switzerland and other parts of Europe.

Here, for the first time, *A. koreicus* was found in Switzerland and in the neighbouring Italian Lombardy region. It was not detected during the surveillance activities in 2012, but only in the 2013 summer season. As no *A. koreicus* eggs were found in 2012, we assume that this species has been introduced de novo although we cannot exclude that it was missed during the 2012 survey. While this is the first report of the introduction of this exotic mosquito species into Switzerland and the neighbouring Italian region, it suggests that the range of *A. koreicus* is generally expanding in Central Europe. Continued surveillance will show if this new invasive mosquito has established a local population and is gaining further ground.

Following the example of other invasive mosquito species, trade with used tyres and domestic plants has been suggested to be the route of entry [14–16]. As the single imaginal specimen from this study corresponds to the morphological form found in Belgium [6] and north-eastern Italy [7], these introductions might all be linked to the same mode of introduction, either at the regional or global level, or even both.

A. koreicus was suspected as a vector of Japanese encephalitis virus [17, 18] and of the dog heartworm *Dirofilaria immitis* [19]. However, its full vector status is currently not resolved and requires further vector competence and field studies.

While the public health relevance of *A. koreicus* is still subject to debate, its presence in the southern part of Switzerland complicates the routine surveillance of *A. albopictus*. It is not possible to distinguish the eggs unambiguously by morphology through a stereo microscope. The eggs need either to be hatched out and reared to late developmental stages for species identification or analysed by technically more sophisticated tools such as DNA sequencing [20] or the MALDI-TOF MS approach applied here [8]. In this study, the full cost per MALDI-TOF MS sample was 10 Euros. This is considerably lower than for PCR. The preparation of the samples is very simple and only takes a few minutes and the analysis itself takes only a few seconds. We appreciate that in a future study more specimens could be processed to gain a more complete picture of the situation.

The expansion and establishment of this, and potentially other, invasive mosquito species in Switzerland and Europe, as a whole, has to be observed carefully. It is important to implement methods that are able to detect a range of invasive species on a routine basis. As such, MALDI-TOF MS is a very useful tool for the identification of mosquito eggs, significantly simplifying surveillance and species-targeted interventions.

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

Authors' contribution
TS conducted the field collections and carried out the laboratory work. TS and PM drafted the manuscript. LE and BFF assisted TS in setting up the field collections. BFF prepared the samples for MALDI-TOF MS analysis. EF, MT and PM supervised the study. All authors reviewed and approved the final version of the manuscript.

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Chapter 4

Susceptibility to current and alternative insecticides in *Aedes albopictus* and *A. aegypti* in Recife, Pernambuco, Brazil and within the Swiss-Italian border region

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4.1 Abstract

Background. *Aedes aegypti* and *Aedes albopictus* are highly invasive mosquito species and have colonised many parts of the world. Both species are vectors of several viruses, including dengue and chikungunya. While *A. aegypti* is the primary vector in the tropics and sub-tropics, *A. albopictus* is increasingly under the public health watch as it has been implicated in disease-transmission in more temperate regions, including continental Europe. Vector control using insecticides is the pillar of most control programmes. When using insecticides there is, however, always the concern of resistance development. We, therefore, set out to assess whether there are any signs of existing or incipient insecticide resistance against currently applied and potentially alternative insecticides in our areas Recife (Brazil) and the Swiss-Italian border region.

Methods. Following World Health Organization guidelines, dose-response curves for a range of insecticides were established for both laboratory-reared and field caught *A. aegypti* and *A. albopictus*. The larvicides included *Bacillus thuringensis* svar. *israelensis* (*Bti*), two of its toxins, Cry11Aa and Cry4Ba, *Lysinibacillus sphaericus*, Vectomax WSP®, a formulated *Bti / L. sphaericus* combination, and diflubenzuron. In addition to the larvicides, the Swiss *A. albopictus* population was also tested against five adulticides, including bendiocarb, DDT, lambda-cyhalothrin and permethrin.

Results. All mosquito populations were fully susceptible to the larvicides tested. Mortality rates in the field-caught Swiss-Italian population showed, however, signs of reduced DDT susceptibility.

Conclusions. Larvi- and adulticides currently used for mosquito control in Pernambuco, Brazil and Switzerland are still effective against the target populations. There are also no signs of incipient resistance against *Bti* as larvae were equally killed by both Cry11Aa and Cry4Ba alone. As both *A. aegypti* and *A. albopictus* display a similar dose-response equal application rates may be used irrespective whether *A. aegypti* or *A. albopictus* is being targeted. The current study also provides an important reference as relatively few insecticide susceptibility studies have been conducted in *A. albopictus*.

Key-words: vector control; insecticide resistance; Biological larvicides; insect growth regulators; *Bacillus thuringiensis israelensis*.

4.2 Introduction

Dengue virus (DENV) and chikungunya virus (CHKV) are mosquito-borne viruses of medical concern in most tropical regions but also increasingly in more temperate regions such as continental Europe. With 50-100 million reported cases each year dengue fever is the most prevalent mosquito-borne disease worldwide (1). According to the World Health Organization (WHO), 2.5 billion people, 40% of the world population, are at risk (2). In the Americas, most dengue cases are reported from Brazil with an estimated 500,000 cases leading to haemorrhagic fever or dengue shock syndrome requiring hospitalisation each year, mostly affecting children (3). In about 2.5% of the cases the outcome is fatal. In Brazil *Aedes aegypti* is the major vector of dengue. .To date, all four DENV serotypes are co-circulating in most of the states (4). The incidence rates in Pernambuco State are particularly high with significant numbers in Recife, the state's capital and largest city. In 2014, the first autochthonous chikungunya cases have were detected in Brazil and by July 2015, a total of 3,554 confirmed autochthonous cases were reported from four states, including Pernambuco (5). In 2015, first cases of Zika virus were also reported from Brazil (6). All the above cases have been linked to *Aedes aegypti*, a known highly competent vector of arboviruses.

A recent outbreak of a mosquito-borne disease in Europe linked to autochthonous transmissions through invasive mosquito species occurred in Ravenna, Italy, in 2007. More than 200 confirmed cases of chikungunya fever were reported and one person died (7). Chikungunya virus (CHKV) is a mosquito-borne alphavirus indigenous to African countries, the Indian subcontinent, and Southeast Asia, where it causes endemic and epidemic fever outbreaks (8). The rapid spread of the disease in Italy demonstrates the vectorial capacity of the local *A. albopictus* population to transmit CHKV. In the same year, two cases of autochthonous dengue fever occurred in Nice, France (9). Following France, Croatia was the second European country that reported locally transmitted dengue cases (10). These outbreaks showed that continental Europe is vulnerable for the transmission of "tropical" arboviruses, particularly in regions where *A. albopictus* and *A. aegypti* are present. In Central Europe *A. albopictus* has a higher importance since *A. aegypti* is currently only present on Madeira and few countries around the Black Sea (Southern Russia, Abkhazia, Georgia) and the Netherlands (11).

A. aegypti and *A. albopictus* are the main vectors of DENV and CHKV worldwide. Both mosquito species have recently shown a large geographical expansion. *A. albopictus*, also known as the Asian tiger mosquito, is ranked as the most invasive mosquito species in the world (11). Originating from South-east Asia, the islands of the western Pacific and the Indian Ocean, it has spread to new continents over the last four decades. Currently it can be found in many regions from North to South America, Africa, Australia and Europe, where its presence has been reported from 20 European countries, including Switzerland (12). Lately, it has been quickly spreading across southern California, USA, which, in this case, has been attributed to climate change. In Brazil, *A. albopictus* was detected in 1986 and has since spread across the country (13). The rapid worldwide expansion of the *A. albopictus* is attributed to its ability of producing egg diapauses that resist desiccation and cold temperatures, allowing this species to be displaced dispersed passively across the globe on board of cargo ships in used tyres and other artificial containers such as plant cuttings (14,15).

As a reaction to the first dengue epidemics in Brazil, a national programme with the aim to eradicate *A. aegypti* (Programa para Eradicação do *Aedes aegypti*-PEAa) was launched in 1996. In 2002 it was replaced by the National Programme for Dengue Control (Programa Nacional de Controle de Dengue-PNCD), adapted to the new challenges and the country-wide expansion of dengue. The main goal of this programme is to fight dengue through integrated control actions, including the use of larvi- and adulticides (16). The main larvicide used in this programme was the organophosphate (OP) temephos, but in 2002, it was replaced by the Biological larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) and the insect growth regulators (IGR), diflubenzuron and novaluron, because of increasing resistance to temephos in several *A. aegypti* populations (17–20). In the city of Recife, the Health Secretary decided to use Bti as the sole larvicide to fight *A. aegypti*. As *A. aegypti* shares the same breeding sites with *A. albopictus* in many urban areas in Brazil, including Recife (21,22), the use of *Bti* by the PNCD equally targets *A. albopictus* larvae.

In Switzerland, *A. albopictus* was found for the first time in the Canton of Ticino in the southernmost tip of the country in 2003. Since then *A. albopictus* has been continuously surveyed (12). In response to increasing densities, the programme was gradually extended in the following years. The estimated *A. albopictus* density was still low, suggesting that individual adult mosquitoes had been sporadically introduced from

neighbouring Italy; however, a sustained population had not yet been established in Ticino. In 2007 this situation changed significantly, when a dramatic increase of positive mosquito surveillance traps was observed, indicating that a local mosquito population had now been established (23). As a reaction on the confirmed arrival and establishment of *A. albopictus* the surveillance in Ticino was expanded and control strategies implemented (24). Nowadays, the monitoring system consists of more than 1000 ovitraps that are analysed bi-weekly (24). Ovitraps are a widely used tool for the surveillance of *Aedes* activity, they are sensitive and characterised by low operating costs (25). The trapping data is used to coordinate targeted applications of insecticides (24). In addition, information campaigns are carried out to raise awareness of the general public in order to avoid having breeding sites on private grounds and to sensitise people on the occurrence of *A. albopictus*. The main weapons of the authorities to fight *Aedes* are the biological larvicide *Bacillus thuringiensis* svar. *israelensis* (*Bti*) for the control of breeding sites and targeted permethrin spraying for the control of adult mosquitoes. Despite these efforts, *A. albopictus* density in the Canton of Ticino has increased over the last years (26).

Bti formulations are widely used (27) and proofed to be effective in controlling mosquitoes in the field (28–30). The toxicity of *Bti* against mosquito larvae is based on crystals produced during bacterial sporulation. The crystals consist mainly of the four pro-toxins Cry11Aa, Cry4Aa, Cry4Ba and Cyt1Aa (31,32). When ingested by the mosquito larvae the crystals are solubilised in the alkaline milieu of the midgut and the released pro-toxins are then activated into toxins in the midgut lumen by gut proteases. The proteases bind to the receptors on the midgut cell membranes, leading to the formation of pores causing cell lysis, septicemia and larval death (33,34). While the *Bti* toxins are highly toxic to the target species due to their synergistic effects when used in combination (32), they remain safe to other organisms due to their specificity (35). *Bti* may be used in combination with *Lysinibacillus sphaericus*, another larvicide that also produces insecticidal crystals. In combination with *Bti*, the toxins display synergistic efficacy in a wide range of mosquito species, including *Aedes* species (27). The IGR diflubenzuron is another insecticide used in both the Ticino and the Brazilian *Aedes* control programmes. Diflubenzuron inhibits the chitin synthesis in the pre-imaginal life stages, inhibiting adult emergence.

This study aimed to examine whether the *A. albopictus* populations in Ticino and *A. albopictus* and *A. aegypti* in Recife that were exposed to over a decade to insecticides are still susceptible to the insecticides currently used. In the Swiss setting, just across the border, in the Italian Como region, no coordinated interventions against the tiger mosquito are in place. This provided the possibility to compare an intervention (Switzerland) versus a non-intervention (Italy) area, side by side. *A. aegypti* populations from Recife were also part of the study considering their epidemiological relevance in that area.

4.3 Materials and methods

Aedes reference colonies

Three *Aedes* colonies were used as susceptibility references for all compounds tested in this study. They were maintained in the insectarium of the Centro de Pesquisas Aggeu Magalhães (CPqAM-FIOCRUZ-PE) in Recife under controlled conditions at 26 ± 1°C, 70% humidity and 12:12 h L:D photoperiod. Larvae were reared in dechlorinated tap water and fed with cat food (Whiskas®, Brazil). Adults were fed on a 10% sucrose solution, and females were additionally fed on chicken blood twice per week. The reference colonies used were: i) Rockefeller: *Aedes aegypti* colony used as an international standard; ii) RecL: *Ae. aegypti* colony established from a large egg sampling from the Recife Metropolitan Region (RMR) that has been maintained since 1996 (38); iii) RecLalb: *Aedes albopictus* colony also established from eggs collected from RMR.

Establishment of *Aedes* field colonies

The field colonies were set from eggs collected in the Canton of Ticino in southern Switzerland (TICINO), the Province of Como in northern Italy (COMO) and from the Recife Metropolitan Region in Brazil, Sítio dos Pintos (SP) and Recife field (RF).

The eggs for the Ticino and Como colonies were provided from an already existing network of 280 ovitraps that were set across the Swiss-Italian border region (Suter et al., *in press*). Wooden slats containing the eggs were removed from the ovitraps every other

week between July and August 2013. The slats were later added to trays filled with de-chlorinated tap water to hatch out the eggs. The first instar larvae were split in equally sized batches to plastic trays and provided with TetraMin fish food (Tetra, Germany). The larval trays were kept in a climate chamber (KBWF 720 E5.2, Binder GmbH, Germany) at 28 °C, 70% relative humidity and a 16:8 h light:dark photoperiod until pupation occurred and any emerged adult was transferred to a 30 cm x 30 cm x 30 cm Bugdom-1 insect cage (Bugdorm, USA). Adults were allowed to mate and provided with water and 10% sucrose solution *ad libitum*. The founder populations of the TICINO and COMO colonies consisted of 520 (380 females and 140 males) and 610 (330 females, 280 males) adult mosquitoes, respectively. The females were then arm-fed twice per week and their eggs collected on filter papers to produce the test population.

In Brazil, the *A. albopictus* (SPalb) and *A. aegypti* (SPaeg) colonies from Recife city were established from eggs collected in 60 ovitraps distributed in district of Sítio dos Pintos as described in Regis et al. (2008) . Upon eclosion larvae were maintained at the insectary of CPqAM-FIOCRUZ (Recife, Brazil), as described above. SPalb and SPaeg colonies were founded with 1,774 (887 females and 887 males) and 3,129 (1,536 females and 1,593 males) adult mosquitoes, respectively. Recife Field (RF), another *A. aegypti* colony representing 45 Recife districts was established from egg sampled using ovitraps that were set according to the recommendations previously described (39). At least 1,000 adults from these samples were used to set up the RF colony. Bioassays were performed on larvae from the first (F_1), second (F_2) or, in exceptional cases, also the third filial generation (F_3).

Test formulations

***Bacillus thuringiensis* var. *israelensis (Bti)* and its individual toxins.** Samples of the lyophilized reference powder IPS82 (Pasteur Institute, Paris) serotype H-14 were used. Individual *Bti* toxins, Cry11Aa and Cry4Ba, were produced in *Bt* acrystalliferous strain 4Q2-81, transformed with plasmids carrying the respective protoxin genes (40). Spore-crystal biomass from each recombinant strain was produced and lyophilized, according to Barros et al. (41). Aqueous suspensions of 5 g/l were prepared and stored at -20 °C

until use. Cry11Aa and Cry4Ba were chosen due to their highest larval toxicity among the major components of the crystal (Crickmore et al 1995).

Lysinibacillus sphaericus. Samples of the lyophilized reference powder SPH88 (Pasteur Institute, Paris), serotype H5a5b strain 2362, were used to prepare aqueous suspensions at 5 g/l that were stored at -20°C until use.

Vectomax WSP (Valent Biosciences Corporation, USA). This product is presented as water-soluble pouches containing a granular formulation which, according to the manufacturer's label combines 4.5% *Bti* (serotype H-14, strain AM65-52) and 2.7% *L. sphaericus* (2362, serotype H5a5b, strain ABTS 1743) fermentation solids, spores and insecticidal toxins as active ingredients (AIs) alongside other ingredients. Samples (batch 179654N8) were used to prepare a stock suspension of 70 g/L (equivalent of 5 g/L of AP) that was further incubated at 25°C for 72 h, in order to allow the release of crystals into the suspension. Aliquots of this suspension were then stored at -20 °C until use.

Diflubenzuron. Analytical standard powder (Sigma-Aldrich Corporation, USA, catalogue co. 45446) was dissolved in acetone to prepare 0.3% (w/v) stock solutions. Aliquots from this solution were stored at -20 °C until use.

Larval bioassays

Susceptibility to *Bti*, its toxins Cry11Aa and Cry4Ba, *L. sphaericus* and Vectomax was analysed following the WHO guidelines for testing larvicides (42). Briefly, groups of twenty 3rd instar larvae were exposed to serial dilutions of lyophilized spore-crystal powder in cups with 100 ml of bacterial suspensions in distilled water, without the addition of food. Five to seven concentrations were tested in each bioassay with three replicates per concentration. A control group was tested using distilled water only. For *Bti*, Cry11Aa and Cry4Ba mortality rates were recorded at 24 h exposure time and for Vectomax and *L. sphaericus* at 48 h. The mortality rates were used to estimate the lethal concentrations of the above compounds to kill 50% (LC_{50}) and 90% (LC_{90}) of the exposed larvae based on general linear models using a probit link function in the statistical software IBM SPSS 10.0 for Windows. Each bioassay was repeated at least three assays performed in different dates.

The efficacy of diflubenzuron to prevent adult emergence was also assessed against 3rd instar larvae following the protocols described in (43,44). Briefly, four to ten concentrations, ranging between 0.2 and 6 µg/l, were tested alongside a negative control containing only the solvent. Larvae were exposed in 8 batches of 10 at each concentration and one batch of 10 for the negative control. The concentrations to inhibit 50% and 90% of the emergence of adult (EI_{50} and EI_{90}) were estimated by Probit analysis. Larvae and/or pupae dead were removed from the bioassay cups in alternate days and the adult emergence was observed up to 30 days. Each assay was repeated three times on different days.

Adult bioassays

In addition to larvicides, the *A. albopictus* TICINO and COMO colonies sampled from the Swiss-Italian border region were also tested for their susceptibility against the four insecticide classes of WHO recommended adulticides. The insecticides evaluated were the carbamate bendiocarb, the organochlorine DDT, the organophosphate malathion, the non-alpha-cyanid pyrethroid permethrin (25:72 cis:trans ratio) and the cyanoid pyrethroid λ-cyhalothrin. The pyrethroids were kindly provided by Syngenta, while the other insecticides were purchased from Sigma-Aldrich, Germany. The bioassays were performed on females of the F_2 generation of the field-sampled eggs following the WHO guidelines for testing adulticides (45). Using a series of insecticide-impregnated filter papers dose-response curves were estimated to determine the lethal dosage (LD) that would kill 50% (LD_{50}) and 90% (LD_{90}) of the TICINO and COMO populations. The filter papers (Whatman no. 1) were impregnated with insecticide in acetone solutions mixed with silicon oil (Dow Corning 556 Silicon) according to (46). The insecticide solutions were serial dilutions with at least five concentrations that yield mortality rates between 0 and 100%. In the test, batches of 17-25 non-blood-fed *A. albopictus* females, aged 2-5 days were introduced into the exposure tubes lined with the insecticide-treated filter papers. The mosquitoes were exposed for 1 hour, then gently blown back into the holding tube and provided with 10% sucrose solution. Following the 24 hours recovery period, the numbers of dead and alive mosquitoes were recorded. Mosquitoes were considered to be alive if they were able to fly. Any knocked-down mosquito, with or without legs and wings, were considered moribund and were

recorded as dead (46). Tests were repeated until a minimum of 100 mosquitoes were exposed per insecticide and concentration, including a negative control.

4.4 Results

In this study, the susceptibility of *Aedes* spp. populations of different origin and different pre-exposure status to control agents was assessed (Table 1). *A. albopictus* populations from the Canton of Ticino in southern Switzerland, the Como area in northern Italy and Recife, Brazil were comparably susceptible to *Bti*. The LC_{50} values ranged from 0.015 to 0.016 mg/l, while the LC_{90} values were between 0.030 and 0.036 mg/l (Table 2). The resistance ratios (RRs) between the *A. albopictus* field (SPalb) and RecLalb reference colonies were less than two-fold, suggesting the field colonies remain to be fully susceptible against *Bti*. Likewise, the LC values for SPaeg and RF, the two *A. aegypti* populations from Brazil were close to those observed for Rockefeller and RecL, the corresponding reference *A. aegypti* colonies (Table 2).

In order to detect early developments in resistance to individual *Bti* toxins, lyophilized powders containing individual Cry11Aa and Cry4Ba toxins were tested separately. Here, too, the LC_{50} values of Cry11Aa and Cry4Ba in the *A. albopictus* and *A. aegypti* field populations were close to those found for the corresponding reference colonies (Table 3). The LCs of the selected Cry toxins were much lower than those of the overall *Bti* crystal, corroborating the *Bti* cocktail to be more effective than individual toxins. The Cry4Ba dose-response curve, in particular, was not suitable for the determination of the LC_{90} since this toxin alone did not achieve high mortality levels, while Cry11Aa mortality curve provided data for the determination of LC_{90} (data not shown). The LC values for *Bti* across *A. albopictus* and *A. aegypti* suggest both species to display a similar level of susceptibility to *Bti*.

In addition to *Bti* the efficacy of another soil bacterium, *L. sphaericus* was tested against *A. albopictus* larvae. Its efficacy spectrum is widely variable across *Aedes* spp., and yet susceptibility data are scarce. Here, the reference powder SPH88, containing crystals with the binary (Bin) toxin from the 2362 strain was evaluated and the LC_{50} and LC_{90} for the RecLalb reference colony were 0.084 and 0.336 mg/l, respectively. The activity

against this colony was only around 10-fold lower than that displayed by *Bti* reference powder (Table x).

The activity of Vectomax, a mixture of *Bti* and *L. sphaericus* crystals, was also investigated to evaluate if this combination of insecticidal crystals could be an effective alternative to control *A. albopictus*. Data from our evaluation showed similar LCs values towards TICINO, COMO and SPalb *A. albopictus* larvae samples (data not shown).

The third control agent tested against immature mosquito stages in the current study was diflubenzuron. Diflubenzuron is used in Switzerland (REF) to control *A. albopictus* yet not in Recife; and hence the efficacy of diflubenzuron was only evaluated against the TICINO colony. Adult emergence was inhibited and the level of susceptibility was not different from the reference colony (Table 4).

A. albopictus populations from the Swiss-Italian border region were also subject to insecticide susceptibility assays against five adulticides (Table 5). Adult mortality after a 1 hour exposure and 24 h holding period to permethrin, λ-cyhalothrin, bendiocarb and malathion at discriminating doses indicated that both *A. albopictus* field populations tested (TICINO and COMO) can be considered as being fully susceptible (99-100% mortality). However, both populations showed suspected resistance to DDT, resulting in 95% mortality. No significant difference in adulticide susceptibility was detected between the *A. albopictus* field population from the intervention area (TICINO) and the population from the non-intervention area (COMO).

4.5 Discussion

In the absence of commercially available vaccines or drugs, dengue and chikungunya, and other arboviroses transmission is currently controlled by vector control. The extensive use of insecticides in many intervention programmes for vector control has raised concerns over the development of insecticide resistance and adverse effects on the environment and human health (47). Genes associated with insecticide resistance have been reported from several mosquito populations, particularly from those vectoring malaria and dengue (48). Due to the dormant egg stages in some *Aedes* species they are more likely to be dispersed passively by global trade than mosquitoes

without this trait. It is, therefore, conceivable that resistance alleles may also spread globally more rapidly. Knowing the insecticide susceptibility status of a local mosquito population is crucial for any intervention programme relying on insecticide use (suggestion Pocquet et al 2014, P&V 7:299). Many programmes have been implemented without previously evaluating the susceptibility profile of the target field populations to the intended control agents. In some cases laboratory colonies have been used as surrogates to establish the susceptibility status, yet such colonies may underestimate the existence of resistance alleles in the field due to founder and bottle neck effects when maintaining laboratory colonies (49).

The biological larvicide *Bti* is known to be effective in reducing mosquito densities in mosquito control programmes. It has a high toxicity to the target species without causing unwanted side-effects in the environment (28,29,50,51). This is particularly important for the control of mosquito species that breed in ecologically sensitive areas where broad-spectrum insecticides cannot be used. Likewise, in urban settings the control of day-active mosquito species like *A. albopictus* and *A. aegypti* by aerosol insecticides is critical because of human exposure to the insecticides. To our knowledge no *Bti* resistance has been reported from mosquito field populations (52–55) and decreased susceptibility to *Bti* is also rare (56–58). Resistance has only been found to single *Bti* toxins in selection experiments under laboratory conditions (57–61).

In the Canton of Ticino in southern Switzerland, the control of *A. albopictus* is mainly based on larval source reduction, either by removing breeding sites or by using *Bti* if the breeding sites cannot be removed (24). Broad-spectrum insecticides are rarely used. Only in exceptional cases permethrin is sprayed on vegetation when mosquito densities cause nuisance to residents in a defined area, or in surroundings from where symptomatic patients with arboviral disease have been reported (24). Nevertheless, the susceptibility status of the Swiss *A. albopictus* population has never been investigated. Here, we performed larval bioassays with *Bti* reference powder IPS82 and two *Bti* toxins Cry11Aa and Cry4Ba. Our study showed no increased tolerance in *Aedes* populations and their susceptibility was similar, regardless of the treatment status of the populations analysed Comparing our results to the findings from other susceptibility

studies (17), it appears that variation of *Bti* susceptibility is narrow. In addition, our data demonstrates that *A. albopictus* and *A. aegypti* are equally susceptible to *Bti*, suggesting that the same application rate may be used where both species are present. In Brazil, *A. albopictus* can be found in many urban environments together with *A. aegypti* and co-existence of both species in these areas is being increasingly reported (21).

Tetreau et al. (59) stated that one of the main reasons for resistance to *Bti* has not been detected in the field is due to the low resolution of bioassays with *Bti* crystals. Previous laboratory studies have shown that exposure to single *Bti* toxins can lead to the selection of resistance, while in combination resistance to *Bti* crystals has not been observed (60–64). In this study their suggested approach was followed and the activity of two individual *Bti* toxins towards larvae was performed to have a more sensitive assay. However, our mosquito test populations were also fully susceptible to Cry11Aa and Cry4Ba. We, therefore, conclude that *Bti* treatments both in Ticino, Switzerland and Recife, Brazil have not exerted a selection pressure strong enough to provoke a differential larval response to these individual toxins. Although no resistance was found in the present study, we advocate using this approach because it may detect incipient resistance and help to prevent or delay insecticide failure.

Like *Bti*, *L. sphaericus* is a naturally occurring soil bacterium that produces a larvicidal toxin (REF, Lacey, 2007 JAMCA). The efficacy of *L. sphaericus* against *A. albopictus* was 8 to 13-fold lower than reported for *Culex quinquefasciatus* (68,69), which is still far better than the LCs to *A. aegypti*, which are 100 to 1000-fold higher (67). The comparison of *L. sphaericus* and *Bti* LCs towards the same *A. albopictus* sample (RecLalb colony) showed that *L. sphaericus* had, in average, LCs only 11-fold higher than *Bti*.

The Biological larvicide Vectomax was also evaluated in this study. Vectomax is a mixture of *Bti* toxins and *L. sphaericus* crystals and combines the *Bti's* advantage of resistance-blocking and the *L. sphaericus'* advantage of longer residuality in a single formulation (70,71). Its activity against *Aedes* spp. was also assessed due to the wide variations of *L. sphaericus* toxicity to this genus (65–67). Our evaluation shows that Vectomax is effective against *A. albopictus*, making it an alternative for the control of immature stages in our study areas. Such conjugated products offer a set five toxins that

can target a wider group of species of medical importance, associated to a low potential of resistance selection. Trials against in different environments have been perfomed to evaluate their field effectiveness (refs 70, 71, Cetin et al 2015 JAMCA, Anderson et al 2011 JAMCA)

Diflubenzuron showed to be another viable alternative to control *A. albopictus* populations according to the low inhibitory concentration found. Nevertheless, the utilisation of this compound in some habitats can have limitations considering its toxicity for some invertebrates (72).

Spray applications of adulticides have also their restrictions but may be required as an emergency intervention in situations of disease outbreaks. In this study, the efficacy of four WHO recommended insecticide classes was tested against *A. albopictus* in the Swiss-Italian border region. Only for DDT an alteration in the mosquito response from these samples was recorded, although the reason behind this finding is unknown since this compound has not been used in mosquito control campaigns in those areas for decades. DDT resistance has also been recorded in *A. albopictus* in Thailand and Japan (73,74), the underlying mechanisms remain unclear.

In summary, the field and susceptible reference colonies evaluated in this study were equally susceptible to the insecticides evaluated. The study implies that the currently used mosquito larvi- and adulticides in Ticino, southern Switzerland and Recife, Brazil are still effective for the control of *A. aegypti* and *A. albopictus*.

Those larvicides have distinct modes of action and this feature is also important to avoid the onset of resistance. Besides the use of insecticides, other strategies showed promising results in decreasing vector densities and could be introduced in integrated mosquito control programs. Natural predators such as the elephant mosquito *Toxorhynchites splendens* or *Cyclopoid* copepods were shown to be very effective in reducing the abundance of *Aedes* larvae in container breeding sites (75,76). Also mosquito control using genetically modified male mosquitoes (77), or the use of *Wolbachia*-infected *Aedes*, able to block virus transmission (78,79), are promising approaches. Several studies also highlighted the benefit of incorporating the community participation in *Aedes* mosquito control programmes (80–83). Often highly productive artificial breeding sites are found on private properties (80) and information campaigns for the elimination of such containers and the correct use of biological insecticides can

result in a significant decrease of the local mosquito population. The cost-effectiveness of such approaches and their long-term success should be evaluated when compared with conventional control methods (84). However, our results also demonstrate the importance of research on insecticide resistance and the need to develop new tools, new insecticides, and innovative strategies to prevent the spread of insecticide resistance in these critical vectors of human diseases.

* The integration of safe and effective larvicides and adulticides in control programs is essential, however, previous studies have shown that *Aedes* control has been a complex task worldwide whose accomplishment depends on the multiples strategies adapted to local conditions. In this scope diversified approaches including biological control, environmental management, and genetic tools should also to be considered as tools to fight *Aedes* species (references of the approaches, 75-83, Regis et al. 2013, PlosOne 8:67682:

4.6 Conclusions

Standard larvicides and adulticides used for mosquito control are effective against the tested *A. albopictus* and *A. aegypti* populations from the studied areas. The susceptibility profiles of the different mosquito populations were similar, despite distinct differences in the interventions. In addition, *A. albopictus* and *A. aegypti* display similar susceptibility levels suggesting the same Biological larvicide applications may be simultaneously applied to target both species where they co-exist.

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4.9 Tables

Table 1. *Aedes* colonies and insecticides evaluated in this study.

Species	Colony	Source	Tested AI						
			Bti H-14	Cry11Aa	Cry4Ba	VectoMax	*L. sphaericus*	Diflubenzuron	Adulticides
A. albopictus	RecLalb	Lab. Brazil	x	x	x	x	x		
	TICINO	Field Switzerland	x	x	x	x		x	x
	COMO	Field Italy	x	x	x	x			x
	SPalb	Field Brazil	x	x	x	x			
A. aegypti	Rockefeller	Lab. Brazil	x	x	x			x	
	RecL	Lab. Brazil	x						
	SPaeg	Field Brazil	x	x	x				
	RF	Field Brazil	x						

Table 2. Toxicity of *Bacillus thuringiensis* svar. *israelensis* (IPS82) against 3rd instar *Aedes albopictus* and *Aedes aegypti* larvae.

Species	Colony	N	LC_{50} (95% CI) [mg/l]	RR	LC_{90} (95% CI) [mg/l]])	RR
A. albopictus	RecLalb	1080	0.009 (0.008-0.011)	1.0	0.028 (0.023-0.037)	1.0
	TICINO	1440	0.015 (0.012-0.018)	1.7	0.036 (0.030-0.060)	1.3
	COMO	1200	0.015 (0.013-0.017)	1.7	0.030 (0.027-0.035)	1.1
	SPalb	1560	0.016 (0.014-0.019)	1.8	0.033 (0.028-0.043)	1.2
A. aegypti	Rockefeller	1620	0.008 (0.007-0.009)	1.0	0.026 (0.021-0.036)	1.0
	RecL	1080	0.013 (0.011-0.015)	1.6	0.032 (0.027-0.039)	1.2
	SPaeg	1200	0.014 (0.012-0.016)	1.7	0.029 (0.025- 0035)	1.1
	RF	1860	0.013 (0.012-0.016)	1.6	0.037 (0.030-0.050)	1.4

LC_{50}: lethal concentration to kill 50% of the mosquito colony; LC_{90}: lethal concentration to kill 90% of the mosquito colony; 95% CI: 95% confidence interval; RR: resistance ratio as compared to the susceptible RecLalb and Rockefeller colony, respectively.

Table 3. Toxicity of Cry11Aa and Cry4Ba toxins against 3rd instar *Aedes albopictus* and *Aedes aegypti* larvae.

Species	Colony	N	LC_{50} (95% CI) [a]	RR[b]	N	LC_{50} (95% CI) [a]	RR[b]
		Cry11Aa			Cry4Ba		
A. albopictus	RecLalb	1,440	0.410 (0.311-0.514)	1.0	1,380	0.595 (0.431-0.787)	1.0
	TICINO	1,500	0.432 (0.335-0.530)	1.1	1,060	0.830 (0.622-1.095)	1.4
	COMO	1,020	0.539 (0.437-0.648)	1.3	1,440	0.483 (0.213-0.839)	0.8
	SPalb	1,140	0.650 (0.517-0.798)	1.6	1,080	0.782 (0.589-1.042)	1.3
A. aegypti	Rockefeller	1,080	0.162 (0.121-0.210)	1.0	1,080	0.331 (0.209-0.492)	1.0
	SPaeg	1,260	0.266 (0.207-0.339)	1.6	1,260	0.685 (0.482 – 0.969)	2.1

[a] Lethal concentration (mg/l) to kill 50% or 90% of the larvae after 24h exposure, and 95% confidence interval.

[b] Resistance ratio (RR) between the LC of the reference colony and that for test sample.

Table 4. Inhibitory effect of diflubenzuron on the development of *Aedes* 3rd instar larvae

Species	Colony	N	IC_{50} (95% CI) [a]	IC_{90} (95% CI) [a]
A. aegypti	Rockefeller	2,000	0.456 (0.352-0.549)	1.197 (1.033-1.448)
A. albopictus	TICINO	1,440	0.376 (0.289-0.462)	1.655 (1.322-2.249)

[a] Inhibition emergence concentrations (mg/l) for 50% and 90% of individuals, mean and 95% confidence intervals.

Table 5. Toxicity of adulticides against *Aedes albopictus* from the Swiss-Italian border region of Ticino.

Adulticide	N	LC_{50} (95% CI)[a]	LC_{90} (95% CI) [a]	WHO diagnostic concentration (%)
Permethrin (25:75 cis:trans)	1,084	0.049 (0.045 - 0.052)	0.115 (0.101 - 0.132)	0.25
λ-cyhalothrin	1,110	0.007 (0.006 - 0.008)	0.014 (0.012 - 0.017)	0.03
Bendiocarb	1,176	0.017 (0.015 - 0.018)	0.027 (0.024 - 0.03)	0.1
Malathion	1,196	0.120 (0.111 - 0.131)	0.362 (0.309 - 0.424)	0.8
DDT	1,261	1.254 (1.139 - 1.379)	3.720 (3.112 – 4.446)	4

[a] Lethal concentration (mg/l) for 50 or 90% of larvae after 24 h exposure, mean and 95% confidence intervals.

Table X. Toxicity of Vectomax® and *Lysinibacillus sphaericus* (Ls SPH88) towards *Aedes albopictus* 3rd instar larvae.

		LC_{50} [a]		LC_{90} [a]	
	No. larvae	**Mean (95% FL)**	**RR[b]**	**Mean (95% FL)**	**RR[b]**
Vectomax®					
RecLalb	1260	0.087 (0.080-0.094)	1.0	0.163 (0.145- 0.190)	1.0
TICINO	1200	0.131 (0.118-0.144)	1.5	0.221 (0.194-0.228)	1.4
COMO	1140	0.077 (0.070-0.085)	0.9	0.136 (0.123-0.155)	0.8
SPalb	1420	0.096 (0.065-0.105)	1.1	0.182 (0.162-0.220)	1.1
Ls					
RecLalb	1200	0.084 (0.070-0.099)	1.0	0.336 (0.239-0.630)	1.0

[a] Lethal concentration (mg/L) for 50 or 90% of larve after 48h exposure, mean and 95% fiducial limits.

[b] Resistance ratio (RR) between the reference colony and the test sample.

Chapter 5

Host feeding patterns of *Aedes albopictus* from Brazil and Switzerland

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5.1 Abstract

Background: The Asian tiger mosquito, *Aedes albopictus* (*Stegomyia albopicta*) is a widespread species that can be found in Asia, the Americas, Europe, Africa and a number of locations in the Pacific and Indian Oceans. This invasive mosquito species is a known vector of several aroviruses, notably dengue and chikungunya. Disease transmissions occur during hematophagy, which is the ability of a female mosquito to acquire blood from a diverse range of animals. While this intrinsic trait is crucial for some biological aspects in female mosquitoes, such as nutrition and egg development, it also enables pathogen transmission to humans and/or animals. Thus, host feeding preference is an important parameter in vector borne pathogen transmission studies, since it helps to understand the species involved in the transmission of diseases.

Methods: Blood meals of field-caught *A. albopictus* females were identified using MALDI-TOF MS and PCR. Both mosquitoes from Ticino, Switzerland and Recife, Brazil have been analysed.

Results: Mammalian blood was dominant in the analysed mosquito blood meals. Human blood was detected most frequently, followed by cows, sheeps, chickens and amphibians.

Conclusions: Consistently to other studies, our results show that *A. albopictus* is highly anthropophilic, even if alternative blood sources are available, underlining its importance as a vector for human pathogens. Also blood from birds was detected, suggesting that *A. albopictus* could potentially act as a bridge vector for avian viruses (e.g. West-Nile virus).

5.2 Introduction

Mosquitoes belong to an important group of vectors that are able to transmit some of the most epidemiologically relevant human diseases, such as malaria, lymphatic filariasis and dengue. Transmission occurs during hematophagy, which is the ability of a female mosquito to acquire blood from a diverse range of animals. While this intrinsic trait is crucial for some biological aspects in female mosquitoes, such as nutrition and egg development, it also enables pathogen transmission to humans and/or animals [1] . Thus, host feeding preference is an important parameter in vector borne pathogen transmission studies, since it helps to understand the species involved in the transmission of diseases. [2, 3].

The Asian tiger mosquito, *Aedes albopictus* (*Stegomyia albopicta*) is a widespread species that can be found in Asia, the Americas, Europe, Africa and a number of locations in the Pacific and Indian Oceans [4-8]. In South America, this species has been documented since the 80s and in Brazil, the first findings were recorded in Rio de Janeiro in 1986. Since then, *A. albopictus* has spread rapidly throughout the whole country. Currently, *A. albopictus* can be found in 24 out of 27 Brazilian States as described by Carvalho *et al*. [6]. In Europe, since its first detection in Albania in 1979, its presence is now reported from 20 countries, including Greece, Italy, France and Switzerland [7]. In experimental studies this species has shown to be competent for the transmission of 26 viruses belonging to various virus families, including, *Flaviviridade*, *Togaviridae*, *Bunyaviridae*, *Reoviridae* and *Nodaviridae.* Yet, *A. albopictus* is being recognized almost exclusively as a chikungunya and dengue vector [4].

In Brazil *A. albopictus* has never been incriminated as a dengue vector, probably due to the presence of *A. aegypti*, which is considered being the main dengue vector, despite some authors have demonstrated that *A. albopictus* populations from Brazil and from other South American countries are highly competent in transmitting dengue -, yellow - and chikungunya fever [9-13]. As a dengue vector of secondary importance, *A. albopictus* surveillance is not a priority of DENV control programmes and information about distribution, density and other mosquito traits are outdated or absent [6, 14].

In Europe, the scenario is different concerning *Aedes* species distribution. Although *Aedes aegypti* is absent in most European countries, probably due to temperate climate, *A. albopictus* is well established and expanding continuously [15]. The establishment of

A. albopictus in Europe raises concern about the transmission of arboviruses between humans or/and animals. In August 2007, autochthonous cases of chikungunya fever were reported in Ravenna (Italy). Epidemiological investigation revealed that this outbreak was associated with the presence of *A. albopictus* [16, 17]. In 2010 two autochthonous cases of chikungunya fever were reported from France and several locally dengue cases were reported again from France and Croatia [5, 17-19]. In Switzerland, *A. albopictus* was first detected in 2003 and is currently firmly established in the South of the country, in the Canton of Ticino [20]. This introduction was probably due to movement through public or private ground transport along highways systems from Italy [7].

Despite the vector potential and medical importance of *A. albopictus*, only few studies have been conducted investigating host feeding patterns of this species in field-collected samples. Studies investigating different *A. albopictus* populations around the world demonstrated that host preferences of this mosquito may vary considerably. *A. albopictus* populations feed preferentially on mammals (especially humans), but they also feed on chickens, other birds, amphibians and reptiles [2, 21-24]. In a study conducted by Kamgang *et al*. [25], it was demonstrated that blood fed *A. albopictus* females, collected in Yaoundé, Cameroon, preferentially fed on humans rather than other mammals. This result is in line with studies conducted in Thailand [2], the United States of America [23], Italy [3], and in La Réunion [26].

In this study, we aimed to identify, by Polymerase Chain Reaction (PCR), the host feeding pattern of field-collected *A. albopictus* from Sítio dos Pintos, a dengue endemic area in Pernambuco State, Brazil, and Ticino, Switzerland, located near Italy. Despite the distribution of this species in both localities, there is no information available on feeding patterns of *A. albopictus* in Brazil or Switzerland. Here, we analyzed blood meals from field caught specimens both from Brazil and Switzerland and evaluated the performance of an established PCR method and a novel approach, using MALDI-TOF MS to identify mixed blood meals.

5.3 Materials and Methods

5.3.1 Study area and mosquito collection

In Brazil, mosquito sample collections were conducted at Sítio dos Pintos, a neighborhood within Recife city (8º 03' S, 34º 52' W, mean elevation: 5 m), Pernambuco State, Northeastern Brazil. The suburban neighborhood stretches over an area of 1.8 km^2 with a population density of 7,276 inhabitants (IBGE, 2010). The area is surrounded by a remnant of the Atlantic Forest, where households are usually distant to each other, and offers a broad diversity of different blood sources. Despite low population density, a previous work showed that both, *Aedes aegypti* and *A. albopictus*, are present in that area. However, *A. albopictus* is much more abundant than *Aedes aegypti* [27].

Mosquitoes were collected using a back-pack aspirator. Sampling was performed within two weeks between May and July 2013 and was conducted both indoors and outdoors. Field-caught mosquitoes were immediately transported to the Entomology laboratory (Centro de Pesquisas Aggeu Magalhães – FIOCRUZ/PE) and sorted by species, sex and gonotrophic stage, using a dissecting microscope. After identification, all mosquito specimens were stored at -80 ^{o}C until further processing.

In Switzerland and Italy, *A. albopictus* females were collected using BG sentinel traps (Biogents, Germany), adding BG lure for attracting *Aedes* mosquitoes. Always two traps were set at ten different locations both in Italy and Switzerland. Ten of them were installed in urban environments and ten in more sylvatic areas. Sampling was performed from middle August until the end of September 2013, during *A. albopictus* population peak time in the Swiss-Italian border region. Traps were installed in the early afternoon and ran for 24 hours. Field-caught mosquitoes were immediately transported to the laboratory (Gruppo cantonale di Lavoro Zanzare, Canobbio, Switzerland) for species determination. After identification, all mosquito specimens were stored at -80 °C until further processing.

5.3.2 Blood meal identification from field-caught *A. albopictus*

Genomic DNA was extracted from dissected abdomens using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Sciences, Germantown, MD, USA). Extracted DNA from field-

caught specimens was eluted in 60 μl of AE elution buffer. In addition to the mosquito specimens, DNA was extracted from crude human, sheep and chicken blood and used as positive controls. Here, extracted DNA was eluted in 100 μl AE elution buffer, diluted in 1:20 for use as PCR templates.

For the identification of blood meals we followed the PCR protocol described by Ezigi *et al.* [28]. This PCR diagnostic distinguishes mammalian from non-mammalian blood meals, while directly identifying various mammalian sources, on the bases of PCR product lengths. A major advantage of the approach is the avoidance of pseudo-gene amplifications that cause false human scores as several blood meal analysis primers match sequences of the mtDNA of *A. albopictus*. Any non-mammalian blood sample may then be further analysed with complementary primer pairs and sequencing, and scores the blood source as either being mammalian or non-mammalian, allowing for further analysis of the non-mammalian samples. Due to the discontinuation of reagents described in Egizi et al. (2013) and to optimize the method for the present specimens, the following protocol was implemented. Reactions of the first round were run in volumes of 25 μl, containing 1x PCR buffer, 200 μM of each dNTP, 50 nM of each primer (CytbVertR1 and UnvRev1C), and 1 unit of Phusion High-Fidelity DNA Polymerase (New England BioLabs, UK) along with 3 μl of template DNA. Cycle conditions were optimized to run at an initial denaturing temperature of 98 °C for 40 s, followed by 25 cycles with a denaturing step of 98 °C for 10 s, an annealing step at 58 °C for 30 s and an extension step at 72 °C for 30s. The final extension was at 72 °C for 10 min. Second round reactions were performed in a final volume of 20 μl, as follows: 1x PCR buffer, 200 μM of each dNTP, 150 nM of Human 741F primer, 500 nM of non-Human F1C primer, 300 nM of UnRev 1C primer, 1200 nM of Pseudoblock primer, and 0.5 unit of Pfu DNA Polymerase (Promega, Fitchburg, WI) along with 1μl of PCR product from the first round. Cycle conditions were optimized to run at an initial denaturing temperature of 95 °C for 2 min, followed by 32 cycles of a denaturing step at 95 °C for 40 s, an annealing step at 61 °C for 40 s and an extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were visualized with Ethidium Bromide on a 1% agarose gel.

Template DNAs that produced no PCR product in the reactions above, were subjected to an alternative protocol with primers for avian mtDNA sequences [29, 30]. PCR assays for birds and/or chicken blood were run with the following modifications: to a

final volume of 25 μl, we added: 1x PCR buffer, 200 μM of each dNTP, 500 nM of each primer (avianF and avian R or galliformF and galliformR), and 1 unit of Phusion High-Fidelity DNA Polymerase along with 3 μl of template DNA. Cycle conditions were optimized to run at an initial denaturing temperature of 98 °C for 30 s, followed by 32 cycles of a denaturing step of 98° C for 10 s, an annealing step of 60 °C for 30 s and an extension of 72 °C for 30s, with a final extension of 72 °C for 10 min. PCR products were visualized with Ethidium Bromide on a 1% agarose gel (Fig. 5.1). To test for contamination, negative controls were employed in all PCR reactions. Positive samples for blood meal identification, except for the human band obtained with Egizi *et al.* assay, were purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Health Care Life Science, USA) and then sequenced in both directions using ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA). Both sequences were assembled into contigs and then checked for quality using the CodonCode Aligner software. Assembled sequences were compared with previously described sequences of known species in GenBank and only the sequences that displayed a similarity above 98% were deemed as the blood meal source.

5.4 Results

In Brazil, mosquito surveillance was conducted in the district of Sítio dos Pintos during two weeks between May and July in 2013. The number of inhabitants varied from 1 to 20 per house and a wide range of different animals is present, such as dogs, cats, horses, goat, donkey, pigs, turtle, cows, birds, chickens, ducks and turkeys. A total of 265 *A. albopictus* specimens were collected by aspiration, however, 140 field-caught females were too desiccated and, then, were discarded from the study. So, in order to increase the chance in detecting positive samples for blood meal identification, we extracted DNA and ran PCR from all the remaining 125 samples, even though when they were visually not blood fed. In forty-eight (38.4%) samples a blood meal could be detected and identified. After sequencing PCR products and comparing the obtained sequences with those available on PUBMED (http://www.ncbi.nlm.nih.gov) and BOLD (http://www.boldsystems.org/), hosts were identified as follows: 15 humans (31%), 15 domestic cows (31%), 10 chickens (21%), 02 other avian species (4%) and 03 mixed bloods (human + cat and 2 human + sheep) (7%). Three positive samples did not match with any species. These results are summarized in Fig. 5.2.

A total of 77 *A. albopictus* females, field-collected in Switzerland, were assayed by PCR. In eight samples (10%) a blood meal could be detected. They were sequenced in order to determine the host profile. After sequencing the PCR products and comparing the obtained sequences with those available on PUBMED (http://www.ncbi.nlm.nih.gov), hosts were identified as follows: 3 humans (37.5%), 2 domestic cows (25%), 2 avian species (25%) and 1 chicken (12.5%). These results are summarized in Fig 5.3.

5.5 Discussion

Our present investigation aimed to analyze the blood feeding pattern of *A. albopictus* populations collected in Sitio dos Pintos (Recife, Brazil) and in Ticino, Switzerland. Despite the importance of *A. albopictus* as a vector for different epidemiologically relevant arboviruses, to date only little data is available on host feeding patterns of this species. Likely because collecting blood-fed females is challenging due to the lack of an efficient trap system for adult mosquitoes. Moreover such studies are laborious, expensive and time-consuming [31]. More simple methods that are based on specifically designed primer pairs [32] or a direct enzyme-linked immunosorbent assay (ELISA) [33] may fail in identifying host species for which the assays are not designed for.

Since *A. albopictus* populations from different localities were shown to feed mainly on mammals [2, 23, 26, 34, 35], our first approach to identify blood feeding patterns was the application of PCR-blocking described by Ezigi *et al.* [28]. This approach allows the identification of human and non-human blood meals and, in addition, the detection of mixed meals, which have important implications for understanding the vector potential of this mosquito. We were only able to detected mixed blood meals in mosquitoes collected from Brazil. In 6% of the analyzed mosquitoes, mixed blood meals have been detected. This finding is consistent to double meal proportion rates from other studies [2, 3, 24]. Samples that could not be identified following the PCR-blocking approach were screened for other blood sources, using previously described avian primers [29, 30]. Although both in Switzerland and Brazil birds as chicken or turkeys were present in the field, we only detected few bird blood meals in mosquitoes, suggesting these *A. albopictus* populations may have limited exposure to avian arboviruses, e.g. West Nile

virus (WNV). This finding is consistent to a study conducted in the Northeastern of the USA [21]. However, the high affinity for mammalian blood highlights the Public Health importance of this species. Our results may indicate that they can also play a greater role in DENV and CHIKV transmission cycles both in Brazil and Switzerland. In summary, our results are consistent with other studies conducted in Brazil [36], Thailand [2], the United States [23], Italy [3], La Réunion [26] and Cameroon [25], showing *A. albopictus* being highly anthropophagic, even if others hosts are available. However, our results also showed that *A. albopictus* from Brazil and Switzerland can also feed on avian species as described in Savage *et al.* [37] and Richards *et al.* [23].

Dengue is still a serious threat in Brazil with more than 22,000 reported cases only in 2015 [38] and the wide distribution of *A. albopictus* in urban and suburban areas poses a real problem for local arboviruse control [6]. In Switzerland, the establishment of *A. albopictus* in Ticino may raises concern about autochthonous transmissions as reported from southern France [39] and Croatia [40]. In addition, recent studies have reported that *A. albopictus* populations from the Americas and Europe are highly competent to transmit all CHIKV genotypes, which highlights the urgent need for continuous entomological surveillance and control measures for *A. albopictus* in countries where the species is present [12, 41]. Our results provide knowledge on the identification of *A. albopictus* hosts and a better understanding of the feeding patterns, a key parameter to recognize the role of a vector species. Our results may help assessing the threat that mosquito species pose to human health and determine their role in disease transmission scenario.

5.6 References

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5.7 Figures and tables

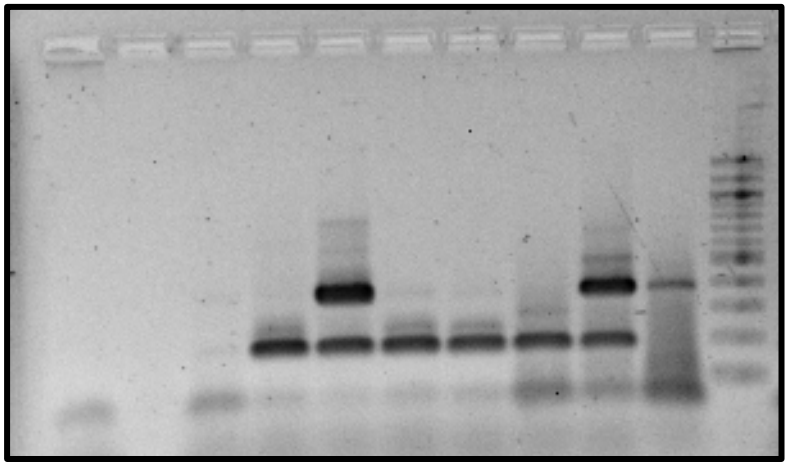

Figure 5.1. Agarose gel visualization of PCR products using PCR-blocking methodology from field-caught *A. albopictus* collected in Sitio dos Pintos, Pernambuco, Brazil.

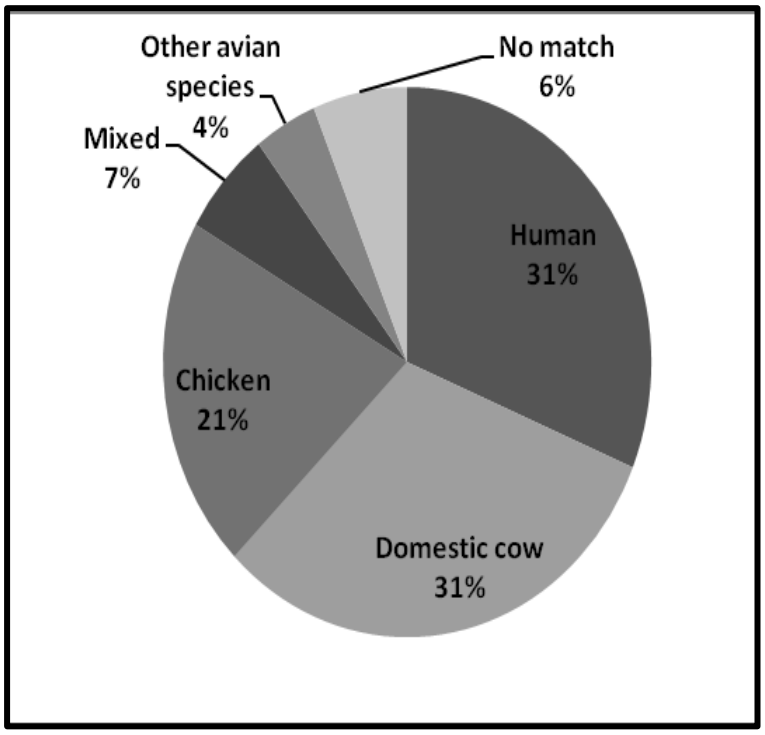

Figure 5.2. Sources of 48 positive samples for blood meal identification in field-caught *A. albopictus* collected in Sitio dos Pintos, Pernambuco, Brazil.

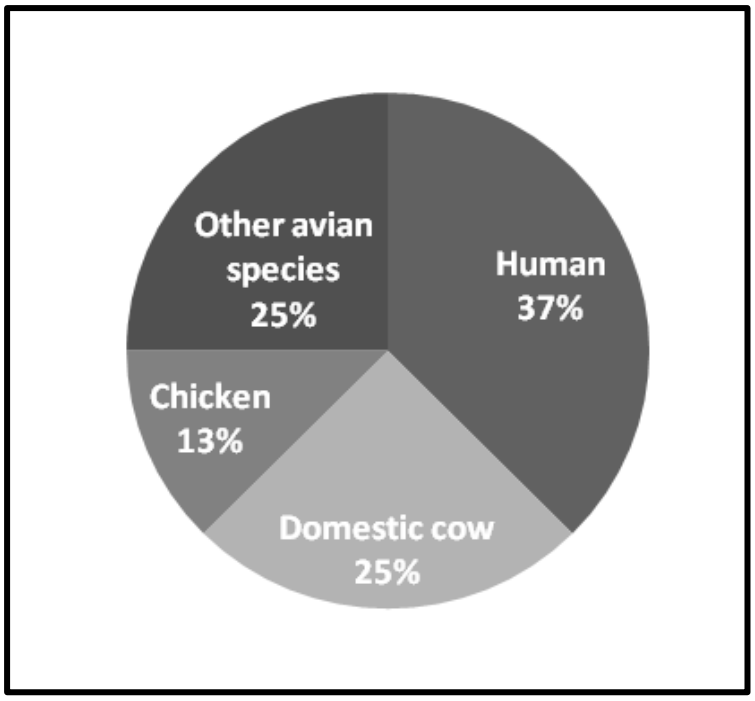

Figure 5.3. Sources of 8 positive samples for blood meal identification in field-caught *A. albopictus* collected in Ticino, Switzerland.

Chapter 6

Nationales Programm zur Überwachung der asiatischen Tigermücke: Zwischenbericht 2014

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In 2014, several sites outside the Canton of Ticino were found positive for *A. albopictus* eggs, some of them repeatedly during the sampling season (Fig. 9). 2014 data clearly show that the south-north axis, the A2 motorway, is an important route of passive distribution for the tiger mosquito. The introduction of mosquitoes is an increasing trend. However, there are, currently, no established *A. albopictus* populations outside the Canton of Ticino.

Abkürzungen

GLZ	Gruppo di Lavoro Zanzare
LMA	Laboratorio di microbiologia applicata (Deutsch: Labor für Angewandte Mikrobiologie)
MALDI-TOF MS	Matrix assisted laser desorption/ionization time-of-flight mass spectrometry
PCR	Polymerase Chain Reaction (Deutsch: Polymerase Kettenreaktion)
Swiss TPH	Swiss Tropical and Public Health Institute (Deutsch: Schweizerisches Tropen- und Public Health-Institut)
SUPSI	Scuola universitaria professionale della Svizzera italiana
SVEG	Swiss Vector Entomology Group
WHO	World Health Organization (Deutsch: Weltgesundheitsorganisation)

6.1 Zusammenfassung

Die asiatische Tigermücke hat sich im Tessin seit ihrer Entdeckung 2003 stetig ausgebreitet und wurde 2013 in der Schweiz erstmals auch nördlich der Alpen nachgewiesen. Da sich die Tigermücke innerhalb von Europa vor allem passiv über Verkehrswege ausbreitet, wurden an Autobahnraststätten, Flughäfen und den Rheinhäfen Mückenfallen an insgesamt 38 Standorten aufgestellt und von Juni bis September 2014 alle zwei Wochen systematisch kontrolliert. Dabei wurden an mehreren Standorten entlang der Autobahn A2, von Chiasso bis Basel, Eiablagen von Tigermücken gefunden. Obwohl es sich bei den Funden nördlich der Alpen um einzelne Einschleppungen und nicht um etablierte Populationen handelte, waren die Funde gegenüber dem Vorjahr häufiger und zeigen nun deutlich die Bedeutung der Gotthardroute für die Verschleppung der Tigermücke von Italien nach Nordeuropa. In weiten Regionen nördlich der Alpen sind die klimatischen Bedingungen für ein Überwintern der asiatischen Tigermücke ungünstig. Trotzdem könnten wärmere Temperaturen während des Sommers zeitlich begrenzte Populationen entstehen lassen. Zudem könnte die Tigermücke auch in wärmere Gebiete, z.B. in die Region Genf, eingeschleppt werden. Dort wären die Bedingungen für ein Überwintern durchaus gegeben. Deshalb empfehlen wir weiterhin eine Überwachung der Tigermücke während den Sommermonaten und die Weiterentwicklung und Umsetzung eines Informations- und Aktionsplans, um bei gehäuften Funden von Tigermücken, gezielt und rasch handeln zu können.

6.2 Einleitung

Die asiatische Tigermücke (Abbildung), *Aedes (Stegomyia) albopictus* (Familie Culicidae; Skuse, 1894), kam ursprünglich aus Südost-Asien und hat sich - vorwiegend durch den globalisierten Handel von Altreifen und Glücksbambus - nach Nordamerika, Lateinamerika, Afrika, Europa und auf mehrere Inseln im pazifischen und indischen Ozean ausgebreitet [1]. Seit 2003 ist diese Mückenart auch bei uns im Tessin heimisch geworden [2]. Hier scheint vor allem die Verbreitung von ausgewachsenen Mücken durch Fahrzeuge entlang der Hauptrouten aus dem Süden eine grosse Rolle zu spielen.

In ihrem ursprünglichen Habitat brütet die asiatische Tigermücke in Baumhöhlen, kommt aber in besiedelten Gebieten auch in anderen, künstlichen Brutstätten wie Gefässen, Abflüssen oder Altreifenlager vor [3][4]. Die Eier, welche über der Wasseroberfläche abgelegt werden, sind trockenresistent und können mehrere Monate, wenn nicht sogar Jahre, überleben. So werden die Eier auch leicht verschleppt, bis sie an einem anderen Ort wieder in Kontakt mit Wasser kommen und eine neue Mückengeneration heranwächst.

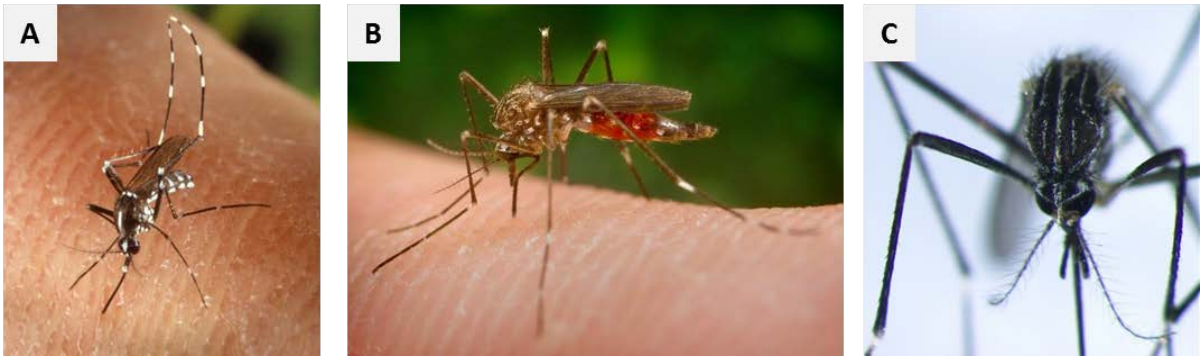

Abbildung 6.1: Invasive Stechmücken in der Schweiz. A: Asiatische Tigermücke, *Aedes (Stegomyia) albopictus*. Quelle: James Gathany, CDC Public Health Image Library **B:** Aisatische Buschmücke, *Aedes (Finlaya) japonicus*. Quelle: James Gathany, CDC Public Health Image Library. **C:** Weibchen der koreanischischen Stechmücke, *A. koreicus*. Quelle: [5].

Neben der Bedeutung als eine der 100 invasivsten Arten [6] ist die asiatische Tigermücke eine Bedrohung aus human- und veterinärmedizinischer Sicht. Weltweit ist diese Mückenart eine wichtige Überträgerin von Viren wie das Chikungunya-, Dengue- oder Westnil-Virus, oder Westnilvirus und wahrscheinlich auch von zahlreichen weiteren Viren wie La Crosse Enzephlitis oder asiatische Enzephalitis aber auch von Fadenwürmern der Gattung *Dirofilaria* [1].

Im Verlaufe der letzten Jahrzehnte haben die Dengue-Fälle weltweit exponentiell zugenommen und Dengue ist inzwischen die wichtigste virale Erkrankung beim Menschen, die durch Stechmücken übertragen wird. Parallel dazu haben auch Meldungen von Denguefieber-Fällen von Rückkehrern in die Schweiz in den letzten Jahren stark zugenommen [7][8]. Gleichzeitig nimmt auch die Bedeutung der asiatischen Tigermücke als Vektor in Europa zu. In Frankreich und Kroatien wurden mehrere Fälle von nicht-importierten Dengue-Fällen, die im Zusammenhang mit der asiatischen Tigermücke stehen, gemeldet [9][10][11][12].

Neben Dengue ist die Tigermücke vor allem ein guter Vektor für Chikungunya. Dies zeigte sich 2007 in Ravenna, Italien, wo über 200 Menschen an Chikungunya erkrankten [13] sowie durch weitere autochthone Fälle der vergangenen Jahre in Frankreich [14][15].

Der Chikungunya Ausbruch in Ravenna, Italien, sowie die diversen Dengue-Fälle in Europa zeigen, dass solche Szenarien auch in der Schweiz denkbar sind, wenn die asiatische Ti-

germücke nicht systematisch überwacht und kontrolliert wird. Es ist davon auszugehen, dass die Einschleppung und Verbreitung der asiatischen Tigermücke (Abbildung) mit steigendem internationalem Handels- und Reiseverkehr, zusammen mit den Folgen der Klimaerwärmung, auch in der Schweiz zunehmen wird [16][17].

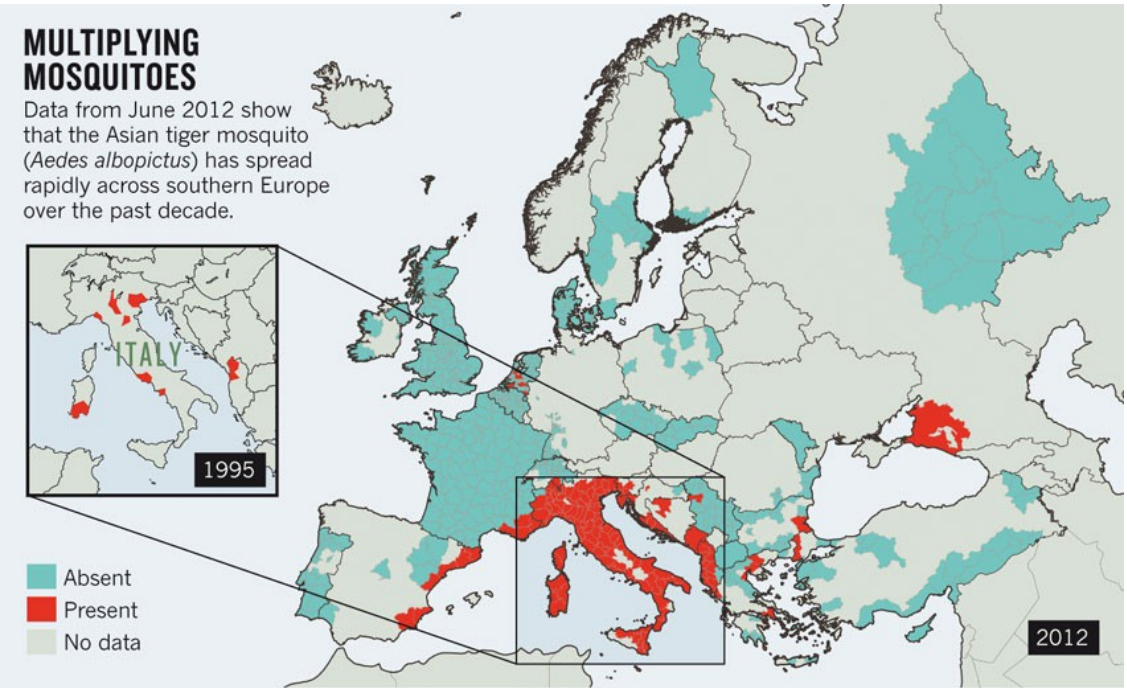

Abbildung 6.2: Expansion der Tigermücke in Europa zwischen 1995 und 2012. Rot: asiatische Tigermücke präsent, Grün: asiatische Tigermücke nicht präsent und Grau: keine Daten vorhanden. Quelle: [18][19].

Seit 2003 wird die asiatische Tigermücke im Kanton Tessin systematisch überwacht und bekämpft. Erst im Jahr 2013 wurde sie im Rahmen eines vom BAFU finanzierten Pilotprojekts auch in der gesamten Schweiz entlang von Autobahnen, an Flughäfen und den Rheinhäfen in Basel überwacht [20]. In dieser Studie wurden an einigen Standorten (Autobahnraststätte Gotthard, UR; Autobahnraststätte Heidiland, SG; Autobahnraststätte Grauholz, BE) in der Schweiz erstmals auch vereinzelte Eiablagen von Tigermücken nördlich der Alpen gefunden. Jedoch war keine Eiablagefalle wiederholt positiv und es konnten keine adulten Tiere gefangen werden. Das deutet darauf hin, dass einzelne Tigermücken als blinde Passagiere mit Autos oder Lastwagen verschleppt wurden, sich bis dahin jedoch keine stabilen Mückenpopulationen etablieren konnten.

Beobachtungen aus dem nahen Ausland zeigen, dass sich die asiatische Tigermücke in Frankreich mit rasantem Tempo aus den Mittelmeerregionen entlang des Rhonetals nach

Norden ausbreitet und bereits in die Nähe von Genf anzutreffen ist [21]. Neben Frankreich und Italien wurde die asiatische Tigermücke auch in grenznahen Gebieten in Deutschland in der Nähe von Basel entlang der A5 [22][23][24] nachgewiesen. In der Schweiz böte auch Genf die Bedingungen für eine Invasion und Etablierung der asiatischen Tigermücke, und in den Regionen Basel, Boden- oder Neuenburgersee würden eingeschleppte Mücken immerhin während den Sommermonaten gut überleben können [17]. Im Zuge des Klimawandels wird zudem vorausgesagt, dass weitere Gebiete ein Überleben der asiatischen Tigermücken potentiell ermöglichen [17].

Neben der asiatischen Tigermücke wurde 2007 die asiatische Buschmücke, *A. (Finlaya) japonicus,* die zweite invasive Stechmückenart in der Schweiz, erstmals nachgewiesen [25] und deren Verbreitung in weiten Teilen des Mittellandes bestätigt [20]. Die asiatische Buschmücke wird in der Bevölkerung aufgrund ihres ähnlichen Aussehens (Abbildung) oft mit der asiatischen Tigermücke verwechselt. Sie kommt ursprünglich aus Ostasien und wurde inzwischen in weiten Teilen Nordamerikas und mehreren Ländern in Zentraleuropa heimisch [26].

Es wird davon ausgegangen, dass die asiatische Buschmücke, wie die asiatische Tigermücke, durch den globalisierten Handel von Altreifen verschleppt wird. Unklar ist jedoch, wie diese Stechmücke in die Schweiz eingeschleppt wurde [25]. Da sie in den kühleren Gebieten Japans und Chinas beheimatet ist, ist sie auch gut an das lokale Klima angepasst. Wie die asiatische Tigermücke, brütet auch sie in bewohnten Gegenden in Gefässen wie herumliegende Dosen, Blumenvasen, etc., wo sie für die Menschen inzwischen zu einer lästigen Plage geworden ist. Im Gegensatz zur asiatischen Tigermücke wird die asiatische Buschmücke jedoch nicht als wichtige Überträgerin von Krankheiten eingestuft [27].

Im Rahmen einer Studie zur Verbreitung der asiatischen Tigermücke an der Grenze zwischen Norditalien und der Schweiz, wurden im Raum Chiasso mehrere Exemplare der koreanischen Stechmücke, *A. koreicus,* gefunden (Abbildung) [28]. Der Vektorstatus von *A. koreicus* ist unklar.

Ziel des vorliegenden Projektes ist es, eine allfällig vorhandene Einschleppung der asiatischen Tigermücke auch nördlich der Alpen sowie im Wallis früh zu erkennen und zu dokumentieren. Da die asiatische Tigermücke sich innerhalb von Europa vor allem passiv über Verkehrswege ausbreitet, haben wir an Autobahnraststätten, Flughäfen und den Rheinhäfen Mückenfallen an insgesamt 38 Standorten aufgestellt und von Juni bis September 2014 alle

zwei Wochen systematisch kontrolliert. Neben der asiatischen Tigermücke wurden die Fallen gleichzeitig auch auf das Vorhandensein der, ebenfalls invasiven, asiatischen Buschmücke, wie auch der kürzlich im Tessin entdeckten *A. koreicus*, analysiert.

6.3 Material und Methoden

6.3.1 Zeitrahmen

Die Erhebungen wurden zwischen Juni und September 2014 durchgeführt. Dieser Zeitrahmen entspricht der Hauptsaison der Tigermücke im Südtessin und Norditalien [29]. Die Mückenfallen wurden in der Woche vom 23. Juni erstmals gestellt und dann alle zwei Wochen bis zum 19. September kontrolliert, so dass insgesamt sechs Kontrollrunden durchgeführt wurden.

6.3.2 Standorte

Da sich die Tigermücke innerhalb von Europa vor allem passiv über Verkehrswege ausbreitet, wurden, wie im vergangenen Jahr [20], Fallen an Autobahnraststätten, Flughäfen und den Rheinhäfen, sowie am Bahnhof Chiasso gestellt. Zudem erhielten wir freundliche Unterstützung vom Kanton Genf. Der Kanton Genf stellte zusätzlich 7 Ovitraps auf dem Stadtgebiet auf (Abbildung A1). Neu haben wir dieses Jahr neben den bisherigen Ovitraps systematisch auch BG Sentinel Fallen für das Fangen von ausgewachsenen Stechmücken eingesetzt.

6.3.3 Mückenfallen

Um das Vorhandensein der Tigermücke nachzuweisen, wurdne an den erwähnten Standorten Eierlegefallen, so genannte „Ovitraps“ [18], gestellt. Ovitraps sind Nachahmungen natürlicher Brutstätten, die trächtige Weibchen zur Eiablage anlocken. Die Fallen bestanden aus einem schwarzen 1.5 Liter Plastikblumentopf, der mit Wasser gefüllt wurde (Abbildung). Die Tigermückenweibchen legen ihre Eier typischerweise auf Oberflächen in Wassernähe ab. In der Falle wurden zu diesem Zweck die Eier auf einem Holzbrettchen, welches aus dem Wasser ragte und zur Eiablage diente, gesammelt. Die Holzbrettchen wurden dann alle zwei Wochen

ausgewechselt und im Labor der Gruppo di Lavoro Zanzare (GLZ) auf Mückeneier kontrolliert.

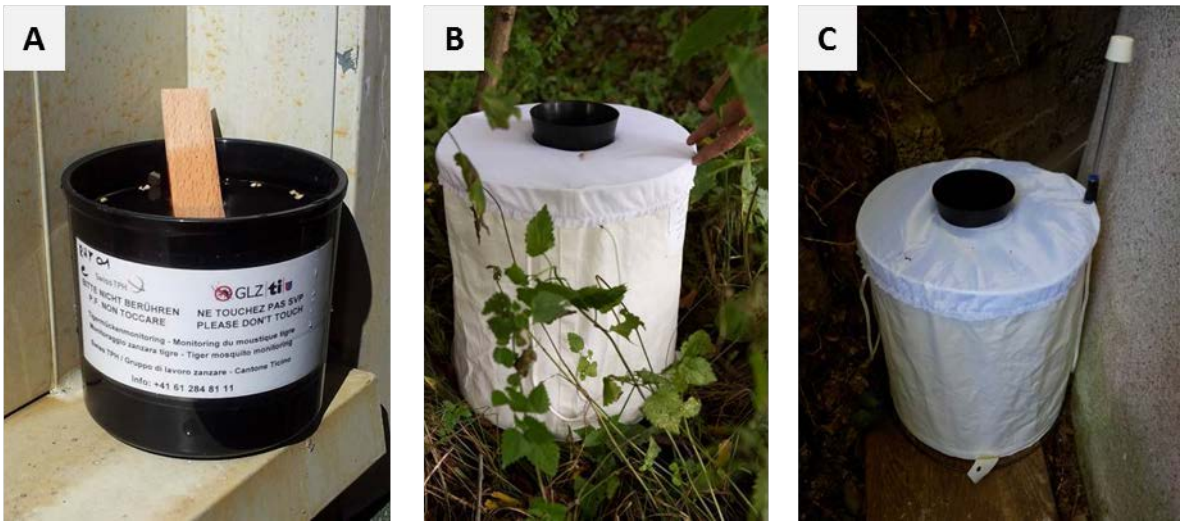

Abbildung 6.3: Eingesetzte Fallentypen. **A:** „Ovitrap“ um Eier von invasiven *Aedes* Arten zu sammeln. Die Weibchen legen ihre Eier auf das aus dem Wasser ragende Holzstäbchen ab. Dieses kann dann im Labor auf Eier untersucht werden. **B:** BG Sentinel Falle um blutsaugende Mückenweibchen einzufangen. Die Falle strömt einen künstlichen Lockstoff aus. **C:** BG Sentinel Falle, die neben dem künstlichen Lockstoff, auch noch mit CO_2 ausgerüstet wurde. Das CO_2 lieferte eine Gasflasche, die über ein Druckventil an die BG Sentinel Falle angeschlossen wurde. Über den Emitter (Stab mit weisser Kappe) strömten dann 200 g CO_2 innerhalb von 24 Stunden aus.

Damit die Fallen selber nicht zu potentiellen Brutstätten wurden, wurde das Wasser mit dem Bakterium *Bacillus thuringiensis* var. *israelensis (Bti)* versetzt. *Bti* tötet potentielle Mückenlarven in den Ovitraps und ist biologisch vollständig abbaubar [30].

Die Ovitraps sind dazu geeignet, festzustellen, ob Mückenweibchen vorhanden sind, die auf der Suche nach einer Brutstätte sind. Diese Fallen sind sehr sensitiv, einfach im Unterhalt und haben sich in der Vergangenheit in ähnlichen Studien bestens bewährt [18][20]. Um eine hohe Sensitivität zu erreichen, wurden an jedem Standort, je nach Fläche, drei bis sechs solcher Fallen aufgestellt.

Das Aufstellen der Fallen am Flughafen Genf, sowie 7 weiteren Punkten in der Stadt Genf, wurde von der Direction Générale de la Nature et du Paysage, Canton de Genève, übernommen. Diese Fallen wurden ebenfalls alle zwei Wochen kontrolliert.

Parallel zu den Ovitraps wurden auch BG Sentinel Fallen der Firma Biogents® (Regensburg, Deutschland) gestellt (Abbildung). Die BG Sentinel Falle eignet sich sehr gut für die Überwachung von Stechmücken, die auf der Suche nach einem Blutmahl sind [31]. Die Mückenweibchen werden mit dem Lockstoff „BG-Lure“ angelockt. BG-Lure wurde speziell für Tigermücken und andere invasive *Aedes*-Arten entwickelt. Um die Attraktivität der Falle zu erhöhen, können diese Fallen zusätzlich mit Kohlenstoffdioxid (CO_2) ausgerüstet werden. Um die BG Sentinel Falle betreiben zu können, braucht es eine Stromquelle. Die Stromquelle kann eine Steckdose oder ein Akku sein. Der Akku muss nach ein bis zwei Tagen wieder aufgeladen bzw. ersetzt werden. Deshalb wurden nur dort BG Sentinel Fallen gestellt, wo der Zugang zu einem festen Stromanaschluss gewährleistet war. So konnten die Fallen parallel zu den Ovitraps kontinuierlich betrieben werden. Insgesamt wurden an 24 Standorten BG Sentinel Fallen gestellt. Davon waren 6 zusätzlich mit CO_2 ausgerüstet.

6.3.4 Identifizierung der Mücken

Alle Holzbrettchen aus den Ovitraps wurden zur Auswertung ins Labor der GLZ in Canobbio bei Lugano gebracht. Dort wurden die Holzbrettchen unter dem Binokular systematisch nach Mückeneiern abgesucht. Wenn auf den Hölzchen Eier vorhanden waren, wurden bis zu fünf Eier pro Gelege entnommen und zur Artbestimmung an die Firma Mabritec AG in Riehen, Basel, geschickt. Dort wurden die Eier mittels Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), einer modernen massenspektrometrischen Methode, auf Artebene analysiert [32][33].

Die Mücken aus den BG Sentinel Fallen wurden zuerst eingefroren und anschliessend unter dem Binokular im Labor des Swiss TPH nach Schaffner [34] und Becker et al. [35] morphologisch bestimmt. Morphologisch ähnliche Exemplare aus einer Falle und derselben Runde wurden in Pools gruppiert. Dann wurde aus jedem Pool mindestens ein Exemplar mit MALDI-TOF MS eingemessen und identifiziert.

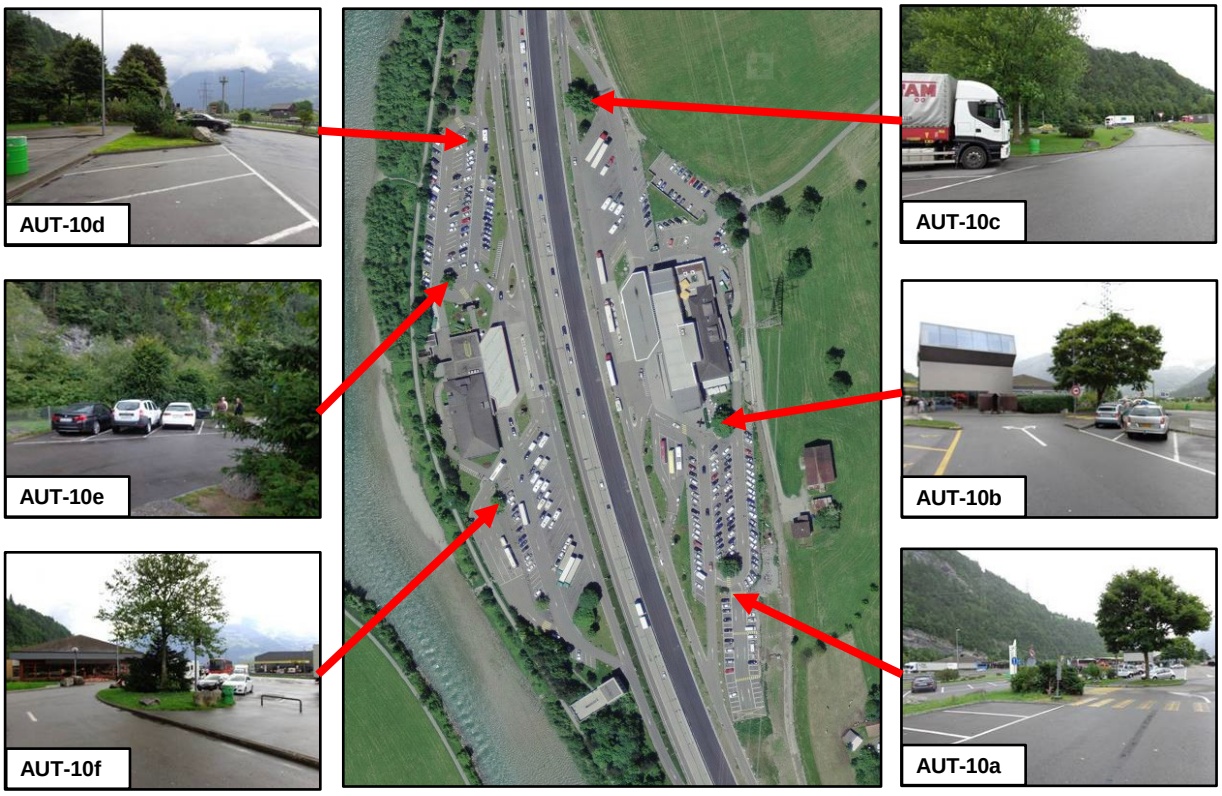

Abbildung 6.4: Ovitrap Fallenstandorte an der Autobahnraststätte A2 Gotthard, Kanton Uri. Bei Rasstätten, die beidseits der Autobahn bedient waren, wurden jeweils sechs Fallen gestellt. Ansonsten wurden nur drei Ovitraps gestellt.

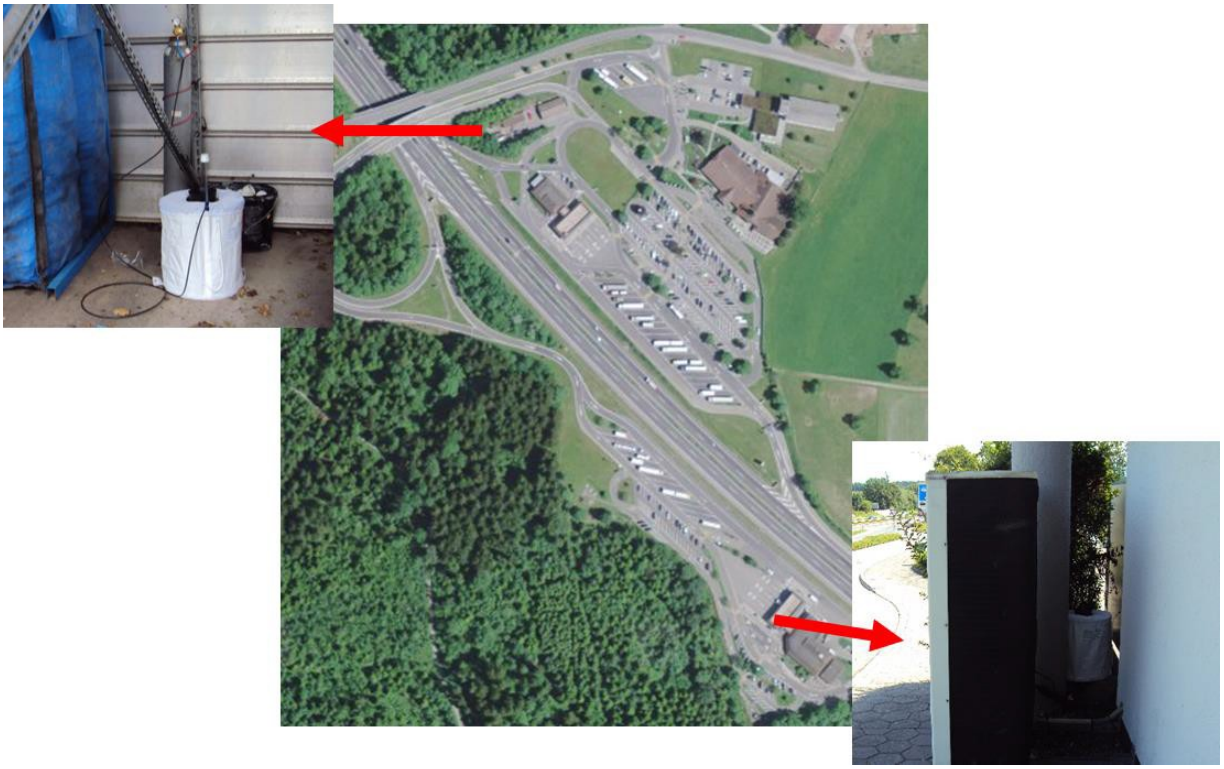

Abbildung 6.5: Beispiel zweier BG Fallenstandorte. Das Beispiel zeigt die Fallenstandorte an der Autobahnraststätte A2 Neuenkirch, Kanton Luzern. Im Falle von Raststätten beidseits der Autobahn wurden wenn möglich zwei BG Sentinel Fallen gestellt, bei einseitigen Raststätten eine Falle.

Tabelle 6.1: Tabelle 1: Fallenstandorte mit Ovitraps. N = Anzahl Ovitraps pro Standort.

Code	Standort	Kanton	Koordinaten	Höhe (m)	N
ARP-01	Flughafen Genf	GE	N 46.23701, E 6.10910	430	6
ARP-02	Flughafen Zürich	ZH	N 47.45399, E 8.57711	432	6
ARP-03	Flughafen Basel-Mulhouse-Freiburg	F	N 47.59356, E 7.54083	254	6
AUT-01	A2 Coldrerio	TI	N 45.84970, E 8.98612	312	3
AUT-05	A2 Bellinzona-Sud	TI	N 46.18211, E 9.00164	227	3
AUT-07	A2 Bellinzona-Nord	TI	N 46.20982, E 9.02753	238	3
AUT-09	A2 San Gottardo-Sud Stalvedro	TI	N 46.52080, E 8.63637	1’064	6
AUT-10	A2 Gotthard	UR	N 46.84612, E 8.63370	460	6
AUT-11	A2 Neuenkirch	LU	N 47.11365, E 8.23129	561	6
AUT-12	A1 Gunzgen-Nord	SO	N 47.31012, E 7.83232	433	3
AUT-12	A1 Gunzgen-Süd	SO	N 47.31015, E 7.84734	444	3
AUT-13	A2 Eggberg	SO	N 47.33595, E 7.82834	549	3
AUT-13	A2 Teufengraben	SO	N 47.33316, E 7.82170	522	3
AUT-14	A2 Pratteln	BL	N 47.52759, E 7.70125	273	6
AUT-15	A1 Kölliken-Süd	AG	N 47.32289, E 8.02166	464	3
AUT-15	A1 Kölliken-Nord	AG	N 47.33007, E 8.03098	438	3
AUT-16	A1 Würrenlos	AG	N 47.43907, E 8.34616	394	6
AUT-17	A1 Kemptthal	ZH	N 47.44858, E 8.70026	503	4
AUT-18	A1 Forrenberg-Nord	ZH	N 47.52667, E 8.73433	468	3
AUT-19	A1 Thurauen-Nord	ZH	N 47.46100, E 9.09423	509	3
AUT-20	A1 St. Margrethen-Süd	SG	N 47.46066, E 9.60297	400	3
AUT-21	A13 Rheintal-Ost	SG	N 47.14597, E 9.50159	455	3
AUT-21	A13 Rheintal-West	SG	N 47.14622, E 9.49989	454	3
AUT-22	A13 Heidiland	GR	N 47.01092, E 9.51217	501	3
AUT-23	A1 Deitingen-Nord	SO	N 47.22889, E 7.62275	423	3
AUT-23	A1 Deitingen-Süd	SO	N 47.22601, E 7.61578	423	3
AUT-24	A1 Grauholz	BE	N 46.99029, E 7.47769	584	6
AUT-25	A1 Rose de la Broye	FR	N 46.83206, E 6.85950	489	6
AUT-26	A1 Bavois	VD	N 46.67460, E 6.56958	555	6
AUT-27	A1 La Côte Jura	VD	N 46.44707, E 6.29995	435	3
AUT-27	A1 La Côte Lac	VD	N 46.44462, E 6.29673	429	3
AUT-28	A9 St-Bernard	VS	N 46.12759, E 7.06026	455	3
RHF-01	Auhafen	BS	N 47.54023, E 7.66176	258	6
RHF-02	Rheinhafen Kleinhünigen	BS	N 47.58450, E 7.58855	249	6
RHF-03	Rheinhafen Kleinhünigen	BS	N 47.58705, E 7.59879	253	6
SBB-03	Bahnhof Chiasso	TI	N 45.84059, E 9.00212	247	6
	Kanton Genf (ohne Flughafen)	GE	Siehe Karte Anhang A		7
Ovitraps insgesamt			161		

Tabelle 6.2: Fallenstandorte mit BG Sentinel Fallen. N = Anzahl Fallen pro Standort.

Code	Standort	Kanton	Koordinaten	Höhe (m)	N
Mit CO_2 ausgerüstete Fallen					
AUT-05	A2 Bellinzona-Sud	TI	N 46.18211, E 9.00164	227	1
AUT-10	A2 Gotthard, Richtung N	UR	N 46.84612, E 8.63370	460	1
AUT-11	A2 Neuenkirch	LU	N 47.11365, E 8.23129	561	1
AUT-22	A13 Heidiland	GR	N 47.01233, E 9.51122	499	1
AUT-27	A1 La Côte Jura	VD	N 46.44720, E 6.29896	437	1
INS-01	Innenhof Swiss TPH	BS	N 47.55564, E 7.57809	279	1
Fallen ohne CO_2					
ARP-02	Flughafen Zürich	ZH	N 47.45399, E 8.57711	432	1
ARP-03	Flughafen Basel-Mulhouse-Freiburg	F	N 47.59356, E 7.54083	254	1
AUT-09	San Gottardo-Sud Stalvedro	TI	N 46.52094, E 8.63467	1’069	1
AUT-10	A2 Gotthard, Richtung S	UR	N 46.84612, E 8.63370	460	1
AUT-11	A2 Neuenkirch	LU	N 47.11365, E 8.23129	561	1
AUT-13	A2 Teufengraben	SO	N 47.33319, E 7.82091	528	1
AUT-14	A2 Pratteln	BL	N 47.52759, E 7.70125	273	2
AUT-19	A1 Thurauen-Nord	SG	N 47.46074, E 9.09383	511	1
AUT-20	A1 St. Margrethen-Nord	SG	N 47.46144, E 9.60363	400	1
AUT-20	A1 St. Margrethen-Süd	SG	N 47.46066, E 9.60297	400	1
AUT-23	A1 Deitingen-Nord	SO	N 47.22862, E 7.62169	425	1
AUT-24	A1 Grauholz	BE	N 46.99003, E 7.47572	584	1
AUT-25	A1 Rose de la Broye	FR	N 46.83180, E 6.85929	494	1
AUT-27	A1 La Côte Lac	VD	N 46.44402, E 6.29471	428	1
RHF-01	Auhafen	BS	N 47.54018, E 7.66166	258	1
RHF-02	Rheinhafen Kleinhünigen	BS	N 47.58450, E 7.58855	250	1
RHF-03	Rheinhafen Kleinhünigen	BS	N 47.58705, E 7.59879	253	1
BG Sentinel Fallen ingesamt					24

6.4 Resultate und Diskussion

Von den insgesamt vom Projektteam gestellten Ovitraps (ohne Kanton Genf) waren jeweils zwischen 136 (84.5%) und 152 (94.4%) bei der zweiwöchentlichen Kontrolle immer noch intakt. Die restlichen Fallen waren z.T. nicht mehr auffindbar, beschädigt, umgekippt, das Holzstäbchen fehlte oder das Wasser nicht mehr vorhanden war.

6.4.1 Identifizierte Mückenarten

Die Eier in den Ovitraps stammten vorwiegend von den zwei invasiven Arten, *A. albopictus* (asiatische Tigermücke) und *A. japonicus* (asiatische Buschmücke). Daneben wurden auch Eier der einheimischen Art *A. geniculatus* gefunden. *A. geniculatus* legt wie die beiden anderen invasiven Stechmückenarten ihre Eier in Baumhöhlen oder künstlichen Wasseransammlungen. Keine der Fallen war positiv für *A. koreicus*.

Die Fundorte der asiatischen Tigermücke und der asiatischen Buschmücke sind in Abschnitt 0 und 0 aufgeführt.

Nur drei Fallen waren positiv für *A. geniculatus*. *A. geniculatus* wurde je einmal an den Autobahnraststätten A13 Rheintal-Ost, A2 Coldrerio und A2 Bellinzona-Nord gefunden.

6.4.2 Asiatische Tigermücke, *Aedes albopictus*

Die asiatische Tigermücke wurde nördlich der Alpen insgesamt sechsmal mit Ovitraps und einmal mit einer BG Sentinel Falle nachgewiesen. Jedoch war keine Falle in zwei aufeinander folgenden Runden positiv. Deshalb wird von einzelnen Einschleppungen ausgegangen.

Neben den Funden entlang der A2 Autobahnraststätten, wurde die asiatische Tigermücke auch einmal am Flughafen Basel-Mulhouse-Freiburg (EuroAirport) nachgewiesen. Da sich die Falle geografisch auf französischem Terrain befand, wurde der Fund umgehend den französischen Kollegen der Brigade Verte du Haut-Rhin mitgeteilt. Diese hatte ebenfalls Ovitraps am EuroAirport gestellt. Jedoch waren weder diese noch weitere gestellte Fallen positiv (P. Bindler, Pers. Komm.), so dass auch hier von einem Einzelfall ausgegangen werden muss.

Mit Ausnahme der Autobahnraststätte A2 San Gottardo-Sud Stalvedro wurde die Tigermücke an sämtlichen Fallenstandorten im Kanton Tessin nachgewiesen (Tabelle , Tabelle und Abbildung). Die Funde im Südtessin bestätigen die Resultate aus dem laufenden Überwachungsprogramm des Kantons [24].

Alle aufgeführten Funde wurden durch MALDI-TOF MS Messungen bestätigt.

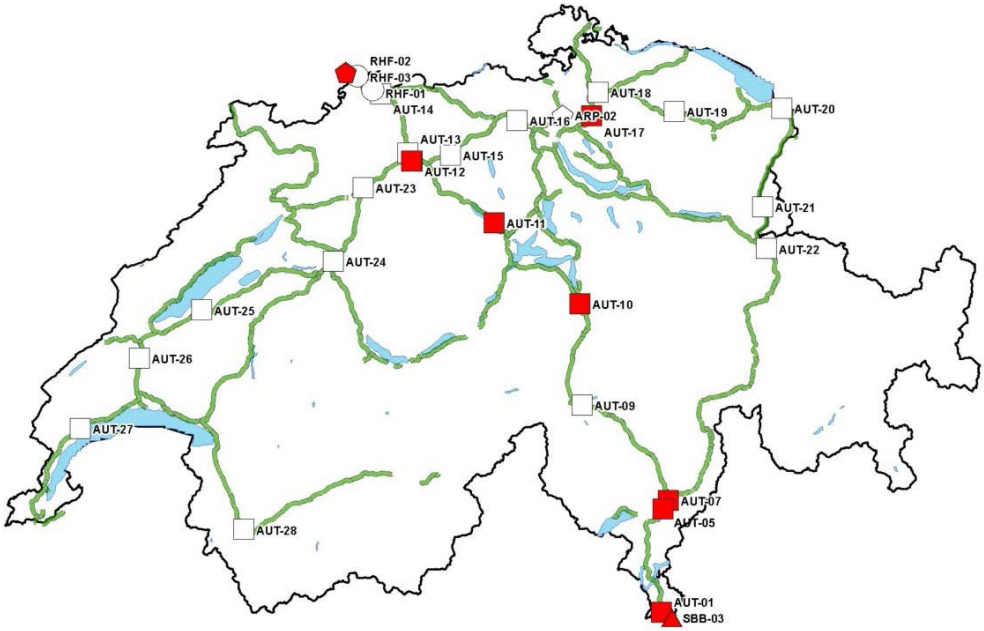

Abbildung 6.6: Fundorte der asiatischen Tigermücke, *Aedes albopictus*. Die rot gefärbten Symbole zeigen die Standorte an, wo mindestens eine Ovitrap oder BG Sentinel Falle einmal positiv war. Die asiatische Tigermücke wurde insgesamt sechsmal nördlich der Alpen gefunden. Die detaillierten Angaben sind in Tabelle und Tabelle aufgeführt. Legende: Vierecke = Autobahnraststätten; Kreise = Rheinhäfen; Fünfecke = Flughäfen; Dreieck = Bahnhof Chiasso; grüne Linien = Nationalstrassennetz.

Tabelle 6.3: Ovitraps, in denen Eier der asiatischen Tigermücke, *Aedes albopictus*, gefunden wurden

Standort	Falle	Kalenderwoche	Anzahl Eier (N)
A2 Coldrerio	AUT-01a	28	118
		30	31
		32	118
		36	99
	AUT-01b	28	23
		32	384
		34	55
		36	154
	AUT-01c	28	36
		30	129
		38	145
A2 Bellinzona-Sud	AUT-05a	28	20
	AUT-05b	32	22
	AUT-05c	28	130
		32	20
		34	27
		36	148
A2 Bellinzona-Nord	AUT-07a	32	30
A2 Gotthard	AUT-10c	28	5
A2 Neuenkirch	AUT-11d	30	15
	AUT-11f	38	80
A1 Gunzgen-Nord	AUT-12b	32	32
A1 Kemptthal	AUT-17a	38	8
Bahnhof Chiasso	SBB-03a	34	11
		36	37
	SBB-03b	32	44
	SBB-03b	34	26
	SBB-03c	30	69
		32	58
		34	89
	SBB-03d	28	21
		30	60
	SBB-03e	38	34
	SBB-03f	38	20
Flughafen Basel-Mulhouse-Freiburg	ARP-03c	34	20

Alle Funde wurden durch MALDI-TOF MS Messungen validiert.

Tabelle 6.4: BG Sentinel Fallen, in denen ausgewachsene asiatische Tigermücken, *Aedes albopictus*, gefangen wurden

Standort	Code	Kalenderwoche	Weibchen (N)	Männchen (N)
A2 Bellinzona-Süd	AUT-05	34	1	0
		36	3	0
		38	2	0
A2 Gotthard	AUT-10	34	0	1

Alle Funde wurden durch MALDI-TOF MS Messungen validiert.

6.4.3 Asiatische Buschmücke, *Aedes japonicus*

Die asiatische Buschmücke wurde praktisch an allen Standorten in der Nordschweiz zwischen den Autobahnraststätten Rose de la Broye und St. Margrethen beobachtet. Das Verbreitungsgebiet überlappt mit früheren Beobachtungen [25][26]. Im Gegensatz zum Vorjahr scheint sich das Verbreitungsgebiet jedoch ausgedehnt zu haben [20]. Es kann davon ausgegangen werden, dass sich die asiatische Buschmücke kontinuierlich in der gesamten Schweiz ausbreitet.

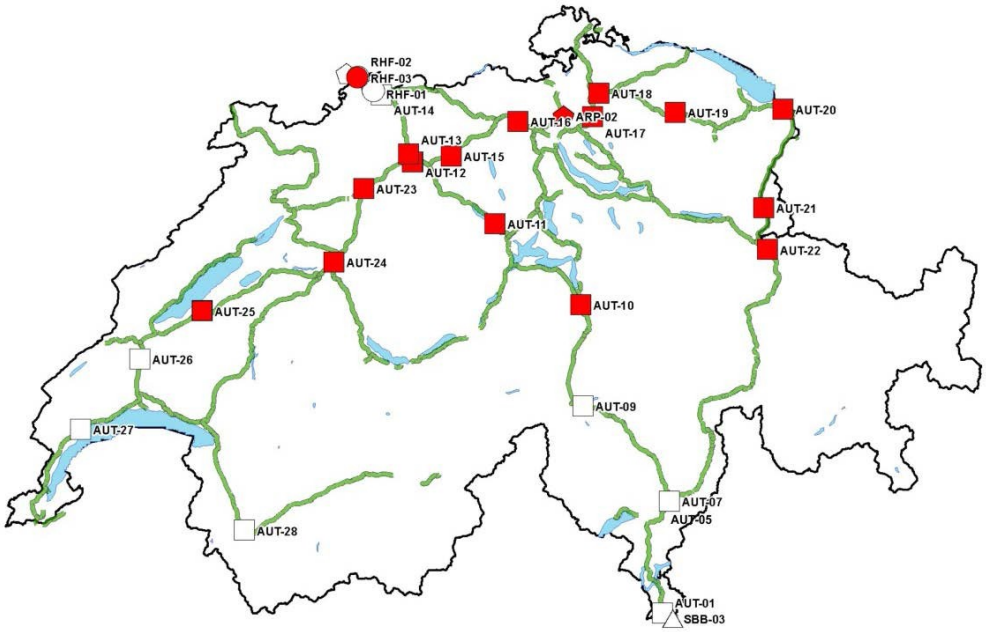

Abbildung 6.7: Fundorte der asiatischen Buschmücke, *Aedes japonicus*. Die grossen roten Kreise zeigen die Standorte an, wo mindestens eine Falle einmal positiv war. Die asiatische Buschmücke wurde praktisch an allen Standorten in der Nordschweiz zwischen den Raststätten Rose de la Broye und St. Margrethen beobachtet. Die zugrunde liegenden Daten sind in der Tabelle 5 enthalten. Legende: Vierecke = Autobahnraststätten; Kreise = Rheinhäfen; Fünfecke = Flughäfen; Dreieck = Bahnhof Chiasso; grüne Linien = Nationalstrassennetz.

Tabelle 6.5: Ovitraps, in denen Eier der asiatischen Buschmücke, *Aedes japonicus*, gefunden wurden

Standort	Falle	Kalenderwoche	Anzahl Eier (N)
Flughafen Zürich	ARP-02a	28	57
		32	61
	ARP-02b	32	44
		34	9
	ARP-02d	38	30
Flughafen Basel-Mulhouse-Freiburg	ARP-03c	34	20
A2 Gotthard	AUT-10a	28	105
	AUT-10b	34	19
	AUT-10c	32	6
	AUT-10e	28	23
		34	1
	AUT-10f	32	119
	AUT-10f	34	49
A2 Neuenkirch	AUT-11e	32	51
	AUT-11f	28	64
		38	80
A1 Gunzgen-Süd	AUT-12a	28	447
		30	54
		32	167
		34	141
		38	74
	AUT-12b	28	57
	AUT-12c	28	108
		30	142
		32	34
		34	29
A2 Eggberg	AUT-13a	32	26
	AUT-13b	30	40
		32	12
		34	60
	AUT-13c	30	67
		32	58
A2 Teufengraben	AUT-13d	36	33
	AUT-13e	28	6
		30	51
		34	16
	AUT-13f	32	43
		38	21

Alle Funde wurden durch MALDI-TOF MS Messungen validiert.

Tabelle 6.5: *Fortsetzung*

Standort	Falle	Kalenderwoche	Anzahl Eier (N)
A1 Kölliken-Süd	AUT-15a	28	292
		30	72
		32	33
		34	85
		36	68
		38	47
	AUT-15b	28	148
		30	90
		32	36
		34	229
		38	261
	AUT-15c	30	44
		38	6
A1 Kölliken-Nord	AUT-15f	28	60
		30	41
		32	42
		34	19
		36	26
		38	56
A1 Würrenlos	AUT-16a	28	155
		30	203
		32	91
		34	198
	AUT-16b	36	50
	AUT-16d	28	35
		32	85
	AUT-16e	28	622
		30	178
		34	82
		38	73
	AUT-16f	28	545
		30	45
		32	58
		38	32
A1 Kemptthal	AUT-17a	34	98
	AUT-17b	32	23
	AUT-17c	30	151
		34	159
	AUT-17d	28	36
		30	90
		34	1

Alle Funde wurden durch MALDI-TOF MS Messungen validiert.

Tabelle 6.5: *Fortsetzung*

Standort	Falle	Kalenderwoche	Anzahl Eier (N)
A1 Forrenberg-Nord	AUT-18a	28	278
		32	23
		36	13
	AUT-18b	28	174
		30	169
		32	18
		36	11
	AUT-18c	28	151
		34	209
		38	4
A1 Thurauen-Nord	AUT-19a	32	14
		38	11
	AUT-19b	28	39
		30	19
A1 St. Margarethen-Süd	AUT-20a	28	42
	AUT-20c	34	10
		36	11
A13 Rheintal-Ost	AUT-21a	38	12
	AUT-21b	32	77
	AUT-21c	32	170
		34	86
		38	1
A13 Rheintal-West	AUT-21e	36	36
		38	59
A13 Heidiland	AUT-22c	38	21
A1 Deitingen-Süd	AUT-23c	28	171
A1 Grauholz	AUT-24a	32	194
	AUT-24b	32	82
	AUT-24c	28	152
		30	99
	AUT-24d	28	124
		30	68
		36	33
	AUT-24e	28	109
		30	297
		32	173
		34	36
		38	17

Alle Funde wurden durch MALDI-TOF MS Messungen validiert.

Tabelle 6.6: BG Sentinel Fallen, in denen asiatischen Buschmücken, *Aedes japonicus*, gefangen wurde

Standort	Code	Kalenderwoche	Weibchen (N)	Männchen (N)
A2 Gotthard	AUT-10	30	1	0
		38	1*	0
A2 Neuenkirch (N)	AUT-11	28	2	0
		30	1	0
		32	2	0
		34	5	0
		36	4	0
		38	1	0
A2 Teufengraben	AUT-13	30	1*	0
		32	1	0
		34	3	0
A1 Thurauen-Nord	AUT-19	32	2	0
		34	1	0
		36	2	0
A1 St. Margrethen-Nord	AUT-20	28	1	0
		30	2	0
A1 St. Margrethen-Süd	AUT-20	34	2	1
		38	5	0
A13 Heidiland	AUT-22	34	1	0
A1 Grauholz	AUT-24	34	1	0
A1 Rose de la Broye	AUT-25	36	1	0
Rheinhafen Kleinhüningen	RHF-02	32	1	0

* Diese Mücken wurden nur morphologisch bestimmt. Alle anderen wurden zusätzlich durch MALDI-TOF MS Messungen bestätigt.

6.4.4 Zeitliche Verteilung der positiven Fallen

Die Anzahl der positiven Eierlegefallen nahm gegen Ende der Überwachungsperiode tendenziell ab (Abbildung und Tabelle). Im Gegensatz zum Vorjahr [20] waren jedoch keine deutlichen Peaks erkennbar. Einerseits könnte dies ein Artefakt sein, welches sich aus einer Kombination aus Anzahl unbestimmter Arten und Anzahl operativer Fallen ergab. Zum Beispiel war die Anzahl der unbestimmten Arten in der ersten Erhebungsrunde sehr gering. Andererseits folgen die Anzahl Einschleppungen nicht zwingend der Aktivitätsperiode der Stechmücken.

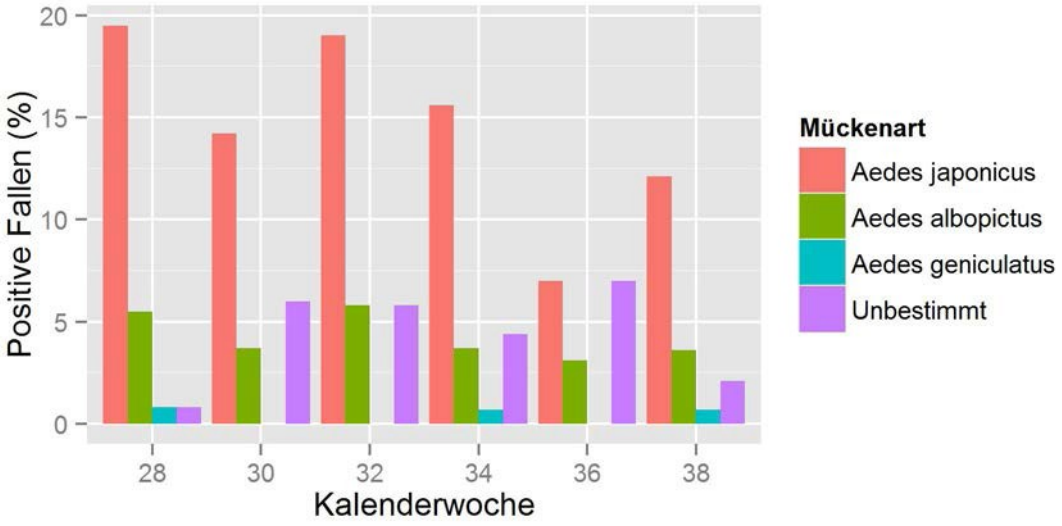

Abbildung 6.8: Zeitlicher Verlauf der Anzahl positiven Ovitraps. Für jede Kalenderwoche wurde die Anzahl der positiven Fallen durch die Gesamtzahl der intakten Fallen geteilt. Die Basis bilden die Werte aus der

Tabelle 6.8: Anzahl der positiven Fallen und Gesamtzahl der Eier pro Art und Kalenderwoche

Mückenart	**Kalenderwoche**	**Intakte Fallen** (N)	**Positive Fallen** (N)	**Eier** (N)
Aedes albopictus	28	129	7	353
	30	142	5	304
	32	145	8	708
	34	141	5	208
	36	138	4	438
	38	143	5	287
Aedes japonicus	28	129	25	4000
	30	142	19	1920
	32	145	26	1740
	34	141	21	1556
	36	138	9	281
	38	143	17	805
Aedes geniculatus	28	129	1	9
	30	142	0	0
	32	145	0	0
	34	141	1	55
	36	138	0	0
	38	143	1	28
Nicht identifiziert	28	129	1	3
	30	142	8	327
	32	145	8	344
	34	141	6	417
	36	138	9	159
	38	143	3	107

6.5 Schlussfolgerungen

Die vorliegenden Resultate zeigen, dass die Tigermücke, wie im vergangenen Jahr, auch 2014 nördlich der Alpen eingeschleppt wurde. Wir gehen davon aus, dass es sich bei den Funden nördlich der Alpen wiederholt um einzelne Einschleppungen und nicht um etablierte Populationen handelt. Jedoch waren die Funde gegenüber dem Vorjahr häufiger. Am häufigsten wurde sie entlang der Autobahn A2 entdeckt. Auch in Süddeutschland wurden zahlreiche Beobachtungen entlang der Bundesautobahn A3, der Fortsetzung der A2, gemacht. Daraus schliessen wir, dass die Gotthardroute eine wichtige Achse in der Verschleppung der Tigermücke von Italien nach Nordeuropa darstellt.

Das Verbreitungsgebiet der asiatischen Buschmücke *A. japonicus* hat sich gegenüber dem Vorjahr ausgedehnt. Wir gehen davon aus, dass sich die asiatische Buschmücke in den Folgejahren noch weiter verbreiten wird.

6.6 Empfehlungen

In weiten Regionen nördlich der Alpen sind die klimatischen Bedingungen für eine Überwinterung der asiatischen Tigermücke ungünstig. Trotzdem könnten wärmere Temperaturen während des Sommers zeitlich begrenzte Populationen entstehen lassen. Zudem könnte die Tigermücke auch in wärmere Gebiete, z.B. in die Region Genf, eingeschleppt werden. Dort wären die Bedingungen für eine Überwinterung durchaus gegeben. Deshalb empfehlen wir, dass die Überwachung der Tigermücke während den Sommermonaten fortgesetzt werden sollte.

6.7 Danksagungen

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A Anhang

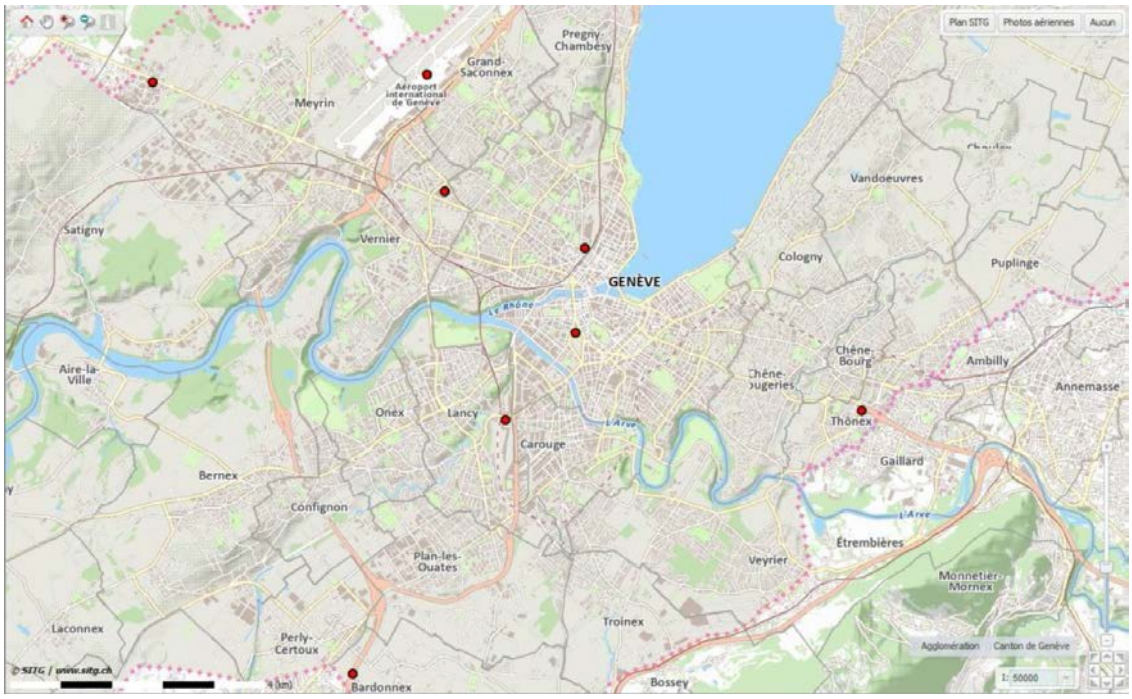

Abbildung A1: Fallenstandorte Kanton Genf. Die roten Punkte markieren die Standorte der Ovitraps, die im Kanton Genf gestellt wurden. Quelle: Corinne Jacquelin, Kanton Genf.

Chapter 7

General discussion and conclusions

7. General discussion and conclusions

7.1 Spatial and temporal distribution of *A. albopictus* in the Swiss-Italian border region

The main focus of this PhD thesis was to evaluate the Ticino *A. albopictus* control approach by comparing mosquito densities in Southern Switzerland (intervention) and Northern Italy (non-intervention). The study was conducted during two consecutive summer seasons in 2012 and 2013 accounting for variations between years. All field work was conducted by the same individual, thereby minimising variations in the methodological approach and guaranteeing high quality standards.

We could clearly show that the abundance of *A. albopictus* is significantly higher on the Italian side of the border as compared to the Swiss side, suggesting the implemented interventions do have an impact. However, other factors may still explain the observed result. The study design did not allow for evaluating individual elements of the Ticino control programme for their contribution in reducing *A. albopictus* abundance. Since in Ticino all *A. albopictus* infested areas are under intervention it was not possible to come up with another study design. It was tried to extract the maximum of information out of the data. By including altitude as an explanatory variable in the model, it contributed significantly to the observed difference in *A. albopictus* egg densities between Ticino and the Italian communities. Although we identified altitude as a confounder in our model, it only negligibly explains the difference between the intervention and non-intervention area.

One possibility to improve the current study design would be to conduct a randomised cluster trial in a previously intervention-free area. All possible intervention combinations (information campaigns, larviciding, adulticiding) would have to be tested in different communities and evaluated by a randomised mosquito density surveillance, following a protocol similar to the one of Vanlerberghe et al. [1]. Different control interventions would have to be assigned to randomly selected communities. The intervention free Como region just across the Swiss border potentially provides a perfect place for such a randomised cluster trial to evaluate the intervention efficacy in the Swiss-Italian border region and would help to better control for potential bias underlying the currently observed differences in *A. albopictus* densities.

Based on scientific evidence for the efficacy of individual or combined interventions, further improvements or adaptations to the current surveillance and control programme in Ticino

could be made. Additional control tools for *Aedes* mosquitoes like large-scale adulticide spraying, the use of insecticide treated curtains, covers for domestic water containers or sugar-baited traps could be tested. Such traps provide an insecticide treated sugar source that attracts and kills mosquitoes. This method showed promising results and could be implemented as an additional intervention tool [2]. Most probably, insecticide fogging would have a hard stand as it likely would not be accepted by the public in Ticino. Fonseca et al. [3] evaluated the effect of large-scale adulticide spraying on *A. albopictus* density in New Jersey, USA. In order to minimise conflicts with residents, the fogging was carried out at night. Pre- and post-trapping of mosquitoes showed a decrease in adult *A. albopictus* in the area but the population had already recovered one week later. As *A. albopictus* is diurnal the impact of insecticide spraying by night is limited. However, large-scale applications of adulticides may help to create buffer zones in a situation of disease transmission [4]. Insecticide impregnated curtains and covers for water containers have been shown promising in reducing mosquito densities [5] and would be worth evaluating for their efficacy in Ticino.

Reviewing the results of our work I think that Italian communities in the border region should be integrated into the Ticino surveillance and control programme. From personal conversations I understand that efforts have previously been undertaken to expand the Ticino control measurements across the border into Italy. Unfortunately, this proved to be rather challenging and eventually has been realised. From other field studies it is known that the effect of interventions against *A. albopictus* in an area is decreased if mosquito control in neighbouring areas is absent. Lacking mosquito control such areas may then serve as reservoirs and allow for continuous re-introduction of mosquitoes into the control area. Therefore, I would strongly suggest reinitiating discussions with the Italian communities about the implementation of a regional and cross-national control program. Although I am wary about the reaction of the Ticino communities, if they were asked to pay money towards an Italian mosquito control programme, Switzerland might actually benefit from a reduction of *A. albopictus* in the Lombardy region. As a consequence, monitoring and control efforts in Switzerland could be reduced at the same time.

GLZ has over a decade of experience in monitoring and controlling *A. albopictus* in Ticino and would be well suited to assist, or perhaps even lead, a cross-border programme. Created in 1995, the “Comunità di lavoro Regio Insubrica” is a Swiss-Italian association that promotes cooperation and integration across the region. The association includes the Italian provinces of Como, Varese, Verbano-Cusio-Ossola, Lecco, Novara and the Swiss Canton of Ticino. The

association could serve as an ideal platform to promote a collaborative project. I do hope the current study helps reinitiating a fruitful discussion and contributes to a constructive dialogue.

7.2 Insecticide susceptibility of the *A. albopictus* field population in the Swiss-Italian border region

The second objective of this dissertation was to investigate the susceptibility of the *A. albopictus* population in Ticino to a range of insecticides, with a focus on those that are currently in use. Fortunately the field-caught mosquitoes tested showed no signs of resistance to any of the insecticides currently in use. Only for DDT we detected a trend towards resistance. However, DDT is currently not used for mosquito control in Switzerland or Italy and therefore this result is not of significant importance. In none of the several studies was *Bti* observed to have a negative impact on the environment and non-target species. The fact that no resistance against *Bti* has been observed makes this insecticide currently an essential tool for mosquito control. However, the treatment of all containers that could serve as breeding sites for *A. albopictus* is extremely laborious and time consuming. In my opinion the incorporation of the public by information campaigns is the most important intervention strategy. This would correspond with findings of other studies aiming to evaluate different intervention approaches.

As the most productive breeding sites are often located on private properties it is crucial to raise public awareness of how to avoid mosquito breeding sites. GLZ has developed an information network that could easily be adapted to the Italian communities. Though this information may also readily be accessed by Italian citizens, there is a pressing need to advertise their existence through the regional authorities and mass media. Existing structures can be used for disseminating information (e.g. newspapers, radio, television, internet, presentations, etc.). As such, information campaigns in the Lombardy region would be relatively easy to implement. Moreover, financial costs would be relatively low. I would strongly suggest evaluating these options together with the neighbouring Italian communities in order to come up with a strategic partnership.

7.3 Host-feeding patterns of *A. albopictus* in the Swiss-Italian border region

The third objective addressed the host-feeding patterns of *A. albopictus* in the Swiss-Italian border region and in Recife, Brazil. In terms of disease transmission scenarios it is important to know the host preferences of the vector to determine which viruses can be transmitted in an area and to assess the threat that a mosquito species poses to human health. During the first field season in 2012, we used sticky traps to collect blood-fed *A. albopictus* females. Sticky traps mimic breeding sites and mosquitoes that try to enter get stuck on plastic sheets that are treated with glue. Mosquitoes get attracted by visual stimuli and water. Likely mosquito density in our study area was too low and additional attractants were needed. The handling in the field was quite complicated and, unfortunately, catch rates were also very low. Therefore we decided to use a different trap system for the 2013 field season. The sticky traps were replaced by BG Sentinel traps (Biogents, Germany). The BG Sentinel traps were equipped with BG Lure, a lure that particularly attracts host-seeking *Aedes* females. Fortunately, the change in trapping system resulted in higher catch rates. As opposed to sticky traps mosquitoes were not covered with glue, thus they were in a better physiological condition, simplifying the analysis.

Initially we planned to use matrix-assisted laser desorption/ionization mass-spectrometry (MALDI TOF MS) for characterising the mosquito blood meals. MALDI-TOF is a soft ionisation technique used in mass spectrometry, allowing the analysis of biomolecules (e.g. DNA, proteins, peptides and sugars) and large organic molecules (e.g. polymers). However, in most of the cases it was not possible to identify the host. Very likely this is due to the fact that mosquitoes partially digested the blood meal or that the proteins already started to degrade. As a consequence PCR was used for the identification of blood meals showing excellent results.

If I was repeating the study I would use human landing catches or a (backpack) aspirator to collect blood-fed females. Following my experience these methods seem more efficient to collected mosquitoes. A disadvantage of these methods would be that the collection is easily biased by the sampling location and the availability of blood sources in the closer surrounding. To compensate for this it would be necessary to collect females in a range of different environments and locations.

7.4 Vector competence of *A. albopictus* in the Swiss-Italian border region

The fourth objective was to examine the vector competence of the Swiss *A. albopictus* population in transmitting the dengue virus serotypes 1, 2, and 3. Similar to the insecticide experiments we used eggs collected in the field (i.e. in the Swiss-Italian border region), to establish a lab colony in Lugano, Ticino. Eggs of the second filial generation were sent to Recife, Brazil to conduct infection studies. At the beginning of the study we were facing several problems. It was necessary to have a climate chamber for simulating climatic conditions present in Ticino. Yet there was an issue with the climate chamber and for this purpose a fridge had to be redesigned to produce a colder climate with lower than ambient temperature. However, due to power outages the colonies were lost multiple times.

Feeding female mosquitoes with infected blood was also very challenging. Unfortunately field mosquitoes were not attracted by the membranes we used for artificial blood feeding. We then tried both, different blood types (human, sheep, chicken) and different membranes. We used artificial membranes (Hemotek Limited, UK), pig gut, chicken- and mice skin. We found that the combination of human blood and mice skin worked best. We additionally used dry ice as a CO_2 source to attract the *A. albopictus* females. During some preliminary experiments at the beginning of the study we detected infected salivary glands, suggesting that *A. albopictus* from Switzerland are competent to transmit dengue virus. Unfortunately we could not confirm this finding in the following experiments. Most probably this was due to low virus titres in the infected blood. To come up with a more conclusive result the experiments ought to be repeated.

7.5 Risk assessment for autochthonous disease transmissions by *A. albopictus* in the Swiss-Italian border region

Considering studies on the dynamic of dengue and chikungunya transmissions, autochthonous transmission by local *A. albopictus* mosquitoes would be possible in the study area, both in northern Italy and Ticino. Following the chikungunya outbreak in Emilia-Romagna in 2007, Carrieri et al. [6] calculated a disease risk threshold in terms of number of eggs per ovitrap above which an arbovirus epidemic may occur. This value is defined as 44.15 *A. albopictus* eggs per ovitrap per week. By the beginning of September, during *A. albopictus* peak time, this value was repeatedly exceeded in our study area. Given an infected person returns from

travelling and gets bitten by a mosquito, local transmissions are possible both in Ticino and the Italian communities just across the national border.

7.6 First finding of *A. koreicus* in Switzerland

During the summer season of 2013 we detected in an ovitraps eggs of *A. koreicus*, a mosquito species yet unknown to Switzerland. In 2014, the number of ovitraps positive for *A. koreicus* increased (Pers. comm. E. Flacio), suggesting that *A. koreicus* is spreading and has possibly also established in Ticino. Within the frame of the routine *A. albopictus* surveillance in Ticino also *A. japonicus* eggs have been found at several locations in 2014 (Pers. Comm. E. Flacio). With the arrival of those two new species there are now three invasive *Aedes* species present in the southernmost part of Switzerland. This complicates the routine surveillance and control program of the Canton of Ticino. It is impossible to distinguish the eggs of these three mosquito species morphologically. As a consequence more laborious and more expensive approaches have to be implemented to assure proper species identification. A possibility would be the use of MALDI-TOF MS for the identification of egg pools. Another possibility would be to rear collected eggs and determine species by morphological characterisation of late instar larvae, pupae or imagines. The *A. albopictus* surveillance programme in Ticino needs to be re-evaluated and needs to take into account the presence of other invasive *Aedes* species. Likely it is necessary to implement adaptations in surveillance and control approaches as a reaction to the different behaviours of these species.

Currently not much is known about the biology of *A. koreicus* and *A. japonicus*. More research is needed on the habitat and host preferences, reproduction, spatial and temporal distribution and their potential of transmitting pathogenic viruses such as dengue and chikungunya in the field. The spread and a possible establishment of these species in Ticino need to be carefully monitored during the following years. *A. koreicus* can tolerate cold winter temperatures and first instar larvae may already be found in early spring [7]. As opposed to *A. albopictus*, the cold winters are not a limiting factor for the establishment and spread of *A. koreicus*, so that this species is very likely to invade the remaining parts of Switzerland and even Northern Europe [8]. In that context it would be useful to establish a lab colony of *A. koreicus* to conduct further studies such as arboviral vector competence studies. It is crucial to know the vector potential of this new invasive mosquito species to assess the risk of affecting public health in Switzerland.

7.7 National Asian tiger mosquito surveillance programme

As described in chapter 6, the Swiss Federal Office for Environment is currently supporting a national surveillance programme for *A. albopictus,* which focuses on potential entry points like airports or harbours and on motorway service stations. The programme is run by Swiss TPH and GLZ. The results of the surveillance program from 2013 and 2014 incl. discussion of results are available in the annual surveillance report.

In summary, we found positive ovitraps also north of the Alps in Switzerland. Both the number of positive ovitraps and the number of eggs per trap increased between 2013 and 2014. It is assumed that these cases were only sporadic introduction rather than established *A. albopictus* populations on the northern side of the Swiss Alps. Remarkably, egg numbers are increasing in Switzerland and *A. albopictus* has also been detected multiple times in Southern Germany at several motorway service stations and parking areas along the German motorway A5 close to Basel. It becomes obvious that the "Gotthard route" is an important route for the passive introduction of *A. albopictus* by road traffic from Italy to northern Europe.

Most regions in northern Switzerland do not provide suitable conditions for the establishment of *A. albopictus*. However, temporal reproduction during the summer season is possible and the species could also be introduced to warmer regions like Geneva or Basel. There the climatic conditions would more likely allow an overwintering of *A. albopictus* and result in the establishment of permanent mosquito populations. Studies on egg mortality in response to cold temperatures have demonstrated remarkable cold-resistance. Experiments showed that 78-99% of eggs from US and Asian temperate *A. albopictus* strains may survive -10°C for 24 hours [9]. A comparison of European temperate and tropical strains of *A. albopictus* and *A. aegypti* demonstrated that minimal survival temperatures for temperate diapausing eggs were -10°C for long-term exposure (12-24 hours) and -12°C for short-term exposure (1 hour), while tropical *A. albopictus* and *A. aegypti* both survived long-term temperatures of -2°C and short-term temperatures of -7°C [10]. Waldock et al. [11] confirmed in their study that temperate, diapausing *A. albopictus* eggs are cold-adapted and able to survive winter nights down to -10°C, although only 10% of the eggs hatched after exposure. In this context one could conduct selective *A. albopictus* rearing experiments whereby mosquitoes are exposed to low temperature schemes. It might be possible to select for dormant egg stages that are even more resistant to cold temperatures than current populations. It is still not clear if the mosquito has really reached its limit of flexibility in terms of low temperatures.

I strongly recommend continuing invasive mosquito surveillance on a national level in Switzerland. As MALDI-TOF MS technique is already implemented routinely in the surveillance project, the expansion of *A. koreicus* can be monitored in parallel. For public health reasons it is advisable to observe the spread and establishment of the three invasive mosquito species *A. albopictus*, *A. japonicus* and *A. koreicus* in Switzerland. Moreover there is a pressing need of developing regional and national action plans. A communication network needs to be implemented and responsibilities to be assigned. Clear action plans as to how to react should mosquito detections increase in an area as well as emergency scenarios in case of autochthonous disease transmission are urgently needed.

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Chapter 8

Appendices

8 Appendices

8.1 Short report (draft): Analysis of vector competence of field-caught *Aedes albopictus* population from Switzerland to different DENV serotypes (Guedes, D.R.D., Suter, T.T.)

8.1.1 Introduction

Vector competence in one of the key determinant for dengue transmission in addition to vector abundance and host preference. Vector competence for DENV may not only vary between species but also among populations of the same species. When compared to *Aedes aegypti* (*A. aegypti*), *Aedes albopictus* (*A. albopictus*) plays a relatively minor role in DENV transmission probably due to differences in host preferences and reduced vector competence (LAMBRECHTS; SCOTT; GUBLER, 2010). It is possible that may still play a role in DENV infections, particularly in areas where *A. aegypti* is absent (ANGELINI et al., 2007; EFFLER et al., 2005; GRATZ, 2004). Understanding some aspects of vector biology such as vector competence should provide a better knowledge of the role of each species in dengue transmission. The intensity of dengue transmission is influenced by parameters concerning vectorial capacity, such as mosquito`s densities, daily survival rate, extrinsic incubation period of the viruses and vector competence (COX; BROWN; RICO-HESSE, 2011; MACIEL-DE-FREITAS; KOELLA; LOURENCO-DE-OLIVEIRA, 2011). Moreover, an important factor underlying experimental infection studies is that susceptibility and viral replication rates may be temperature-dependent. Westbrook *et al*. (2010) showed that climate factors, such as low temperature experienced at the larval stage can increase the competence of adult females to vector arboviruses. As a consequence, knowledge over vector competence of *Aedes* populations specific to each location in relation to more realistic climatic conditions would allow estimating the level of risk of dengue transmission more accurately.

Vector competence has been evaluated by different techniques including immunofluorescence and quantitative RT-PCR (qRT-PCR). Although immunuofluorescence has been used in some virus-vector interaction studies (BENNETT et al., 2002; LOURENCO-DE-OLIVEIRA et al., 2004; VAZEILLE et al., 2003), this technique is a qualitative method and thus is not applied for virus quantification. In order to quantify viral nucleic acid in specific tissues to study viral infection, replication and dissemination, studies have been used qRT-PCR technique, which is a reliable and sensitive method used for this purpose (ARMSTRONG; RICO-HESSE, 2003;

BAE et al., 2003; KEMPF; BLAIR; BEATY, 2006; RICHARDSON et al., 2006; SALAZAR et al., 2007; VANLANDINGHAM et al., 2004; ZHANG et al., 2010).

In this work, we proposed to analyze natural populations of *A. albopictus* (from Brazil and Switzerland) with regards to their susceptibility level to different DENV serotypes. Our aim was to measure how these populations respond to the different DENV serotypes. Elucidation of dynamics replication and tropism of Dengue virus in field-relevant *Aedes* sp. would provide a better understanding of some factors that condition more accurately dengue epidemiology and local epidemic potential.

8.1.2 Material and methods

Aedes albopictus rearing

In order to establish mosquito populations, *Aedes albopictus* eggs were collected from ovitraps, adapted from Fay and Eliason model (regis et al, 2008), that were distributed in the Canton of Ticino, Switzerland. In the insectary of the Department of Entomology at Centro de Pesquisas Aggeu Magalhaes (CPqAM), eggs were hatched for larval eclosion and, then, mosquitoes were raised according standard protocols. All the experiments with field mosquito population described here were performed with F2-3 generations. A laboratory colony was also included. The *Aedes albopictus* laboratory colony was collected from the municipality of Moreno ($08^{o}07'07''S$ $35^{o}05'32''W$) and has been maintained in the insectary over eight years. Mosquitoes were maintained in an insectary at 26 ± 2^{o} C, a relative humidity of 70-80% and a 12:12 hour light-dark cycle until further usage.

Viruses

A low passage of DENV1 (GenBank: EU259529), DENV2 (GenBank: EU259569) and DENV3 (GenBank: EU259607) strains, isolated from dengue patients during epidemics in Recife in 1997, 1995 and 2002, respectively, were used in this study. Viral stocks were prepared as follows: the *Aedes albopictus* cell line (C6/36) was grown to 90% confluency in $75cm^2$ flasks using grown media (Leibovitz L-15 medium supplemented with 5% of fetal calf serum and 1% penicillin/streptomycin). Prior to virus inoculation, the grown media was completely removed and, then, dengue viruses were inoculated at a multiplicity of infection

(MOI) of 1 in 2 ml of maintenance media (Leibovitz L-15 medium supplemented with 2% of fetal bovine serum and 1% penicillin/streptomycin) and incubated for 1 hour at 28^{o}C. Flasks were then supplemented with 28 ml of maintenance media and maintained at 28^{o}C where they were monitored daily until the presence of cytopathic effect in more than 90% of the cells. Viruses were harvested and individually aliquots of 0.5-1.0 ml were stored at -70^{o}C.

For oral challenges, an aliquot (250 µl) of each DENV stocks were used to infect 80-90% confluent C6/36 cells at a MOI of 0.01 in 25 cm^2 cell culture flasks separately. Cells were incubated at 28^{o} C for one hour to allow virus adsorption. After addition of 10 ml of Leibovitz L-15 medium (GIBCO, Invitrogen, Grand Island, NY) supplemented with 2% of fetal calf serum and 1% penicillin/streptomicin, flasks containing infected cells were then incubated at 28^{o} C and monitored daily until the appearance of cytopathic effects. Flasks containing non-infected C6/36 cells were used as negative control in oral infection experiments.

DENV oral infection

Ten to fifteen day-old *Aedes albopictus* field population and laboratory colonies were challenged with DENV1, DENV2 and DENV3 strains. Briefly, 200-250 *Aedes* sp. female mosquitoes, one week old were deprived of sugar and water 24 hours prior to oral infection. To prepare infectious blood meal, which was provided to the mosquitoes using artificial feeders, cells and supernatant were mixed with defibrinated blood sheep at a ratio of 1:1. The control groups were mosquitoes fed on uninfected C6/36 cells also mixed with defibrinated sheep blood. Blood meal was maintained at 37^{o} C by circulating warm water. Mosquitoes were allowed to feed for 45-60 minutes with final titers that varied as follows: DENV1 (7.5 x 10^4 and 5.5 x 10^4 FFU/ml, 1st and 2nd trial, respectively), DENV2 (2.0 x 10^4 and 1.75 x 10^4 FFU/ml, 1st and 2nd trial, respectively) and DENV3 (5 x 10^4 and 1 x 10^5, 1st and 2nd trial, respectively). Fully engorged female mosquitoes were selected and transfer to another cage and maintained at the BSL3 insectary during 15 days under different climate conditions. In order to simulate Brazilian climate conditions, mosquitoes were maintained in a constant temperature of 27 ^{o}C, and in order to simulate Swiss climate conditions, mosquitoes were maintained for 16 hours at 27 ^{o}C and then 8 hours at 16 ^{o}C.

Mosquito dissection and RNA extraction

Midguts and salivary glands from up to twenty mosquitoes per climate condition and per population from control and infected groups were dissected in cold PBS at 7 and 15 days post-infection (dpi). After dissection, tissues were placed individually in a 1.5 ml RNase-free microcentrifuge tubes that contained 300 µl of a mosquito diluent (LAMBRECHTS et al., 2009). Samples were snap frozen and immediately stored at -80^{o} C.

Before RNA extraction, tissues were homogenized in mosquito diluent using autoclaved pestles and automated homogenizer. Total RNA was extracted from 100 µl of viral stocks and tissues using Trizol reagent (Invitrogen) according manufacturer's instructions and eluted in 30 µl of DNase/RNase free water (Gibco). Extracted RNAs were treated with DNase TURBO (Ambion) in order to avoid DNA contaminations and then quantified by NanoDrop 2000 (Thermo Scientific).

One-step SYBR green real-time RT-PCR

To obtain a standard curve for quantitative real-time PCR, serial dilution of known copies of dengue virus RNA is always required. For this purpose, dengue virus RNA standard for each serotype was obtained by in vitro synthesis of RNA transcripts using PCR products of each dengue virus serotype. In vitro transcription was done by using MAXIscript in vitro transcription kit (Ambion,Cat. no. 1314) as described by Kong (Kong et al. 2006). After purification of RNA transcripts using Phenol:Chloroform:Isoamyl Alcohol (Invitrogen) and Sodium Acetate (Ambion), the amount of the IVT generated dengue RNA fragments were determined by NanoDrop reading and converted to molecular copies by using the formula described in Kong et al. (2006). RNA stock solution for each dengue virus serotype was diluted 10-fold serially using DNase/RNase free water and stored frozen at -80^{o} C.

The RNAs of mosquitos' midguts and salivary glands infected with DENV1, DENV2 and DENV3 strains were detected by SYBR Green I-based quantitative RT-PCR using QuantiTect SYBR Green PCR kit (QIAGEN). For that, primers that amplified a 104 pb region of the DENV non-structural protein 5 (NS5) described by Kong et al., 2006 were used. PCR reactions contained: 1X SYBR Green Master mix, 0.2 µM of forward and reverse primers, 0.2 µl RT enzyme, 50 ng of RNA and treated water to complete 25 µl of the total volume. Reactions were performed in 96 well microplates and placed in an ABI 7500 Thermocycler as

follows: 50° C for 30 minutes, 95 ° C for 15 minutes followed by 40 cycles of 94 ° C for 15 seconds, 58 ° C for 30 seconds and 72 ° C for 30 seconds. After the extension, a melting curve was included to confirm product specificity. The amount of virus in each sample was calculated by cycle threshold (Ct) values from the standard curve included in each PCR plate. Negative controls consisted of reaction with control samples (mosquitoes that fed on non-infected blood meals) while positive controls was a DENV1, DENV2 and DENV3 mosquito sample previously assayed by qRT-PCR. All samples and DENV standards were performed in duplicate. Viral genome was compared during all time points using Student's t-test and one-way ANOVA using GraphPad Prism version 4.0.

Virus titration

Viral titers were established using protocol described by Santos *et al* (SANTOS et al., 2013). C6/36 cells were grown to confluent monolayers (80-90%) in 24-well plates 48 hours prior to virus infection. Then growth media was removed in the 24-well plates and 100 µl of each 10-fold serial dilution of viruses was added for 1 hour and then overlaid with a mixture of 50 ml of carboxymethyl cellulose, 48 ml of maintenance media and 2 ml of FCS. After 5 days of incubation at 28° C, the overlay was removed from each well by aspiration, cells were washed twice with cold 1X PBS and then fixed with 30% of cold acethone for 13 minutes. The fixative was removed and cells were allowed to dry for 24 hours at 37° C. Monoclonal primary antibody against dengue viruses diluted 1:50 was added in each plate for 1 hour at 37° C and then washed twice with 1X PBS. An anti-mouse horseradish peroxidase (HRP)–conjugated antibody (Sigma, cat # A6926) diluted 1:500 were used as a secondary antibody. Foci were revealed after the addition of 1 tablet of AEC (3-Amino-9-ethylcarbazole, Sigma Cat# A 6926) substrate dissolved in a mixture of 6 ml of N,N-Dimethylformamide (Sigma), 200 µl of 30% hydrogen peroxidase and 93.8 ml of substrate buffer (148 ml of Acetic acid 0.58% V/V, 352 ml of Sodium acetate 1.36% W/V and 500 ml of distilled water). Viral titers were determined by counting foci.

8.1.3 Preliminary results

A total of 960 samples were analyzed in the present study. These samples, including midguts and salivary glands, were collected from the DENV infection experiments with serotypes 1, 2

and 3. These samples were then subjected to RNA extraction with subsequent treatment with DNase. Considering that salivary gland is the organ related to vector competence, they were first analyzed by qRT-PCR. From the DENV2 infection experiments we analyzed a total of 232 salivary glands and with regards to DENV3, we analyzed a total of 180 salivary glands. As a preliminary result, it was not possible to identify the presence of viral particles in the samples of salivary glands of feeding experiments with DENV2 and DENV3. This may have happened due to the low titer used during artificial bloodfeeding (DENV1: 7.5 x 10^4FFU / ml in the 1st trial and 5.5 x 10^4 FFU / ml in the 2nd trial; DENV2: 2.0 x 10^4 FFU / ml in the 1st trial and 1.75 x 10^4 FFU / ml in the 2nd trial and DENV3: 5 x 10^4 FFU / ml in the 1st trial and 1 x 10^5 FFU / ml in the 2nd trial). The salivary glands collected from the feeding experiment with serotype 1 and midguts for all the three serotypes are still under analysis.

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8.2 Interactions with the press

During the last years the Asian tiger mosquito caused a lot of sensation in the general public and media in Switzerland. In 2012 and 2013, when our field study was carried out, we had several contacts to media, both in Italy and Switzerland. In a first phase we needed permissions of all Italian communities to conduct our field samplings on their territory. It was very important to inform the general public about our project in order to minimise problems in the field. Those meetings were always very positive and fruitful. As a consequence of our activities in early summer 2012, the province of Como organised a meeting with the local press in front of the Como city wall. Retrospectively this was very important and facilitated our work tremendously. People knew our faces and the aim of our study. Mostly we were very welcome and also allowed to enter private properties.

During the field season 2013, the Ticino television broadcast RSI (Radiotelevisione Svizzera) made a documentary (Faló) on *A. albopictus* in Ticino. In the frame of that we could also present our project. Later on in that season, reporters of the Swiss newspaper “Neue Zürcher Zeitung” (NZZ) accompanied us in the field and documented our study.

In 2014, the tri-national television broadcast 3sat filmed a documentary on my person with a focus on the work we did in Ticino and northern Italy.

All those interactions with the press were very positive and I learned a lot about interactions with media and communication. In the following sections all the mentioned articles and television programmes are listed and presented.

8.2.1 *Interactions with the north Italian press (Corriere di Como, Corriere della sera)*

RASSEGNA STAMPA
COMUNE DI COMO

EVENTI - 3061 ANNO XXXIII

Edizione del 14 GIU. 2012

CORRIERE DI COMO

La zanzara tigre ha le ore contate Sono arrivate le "trappole" svizzere

Verrà anche analizzato il sangue delle "vittime"

«Conosci il tuo nemico e saprai come sconfiggerlo». Una frase trita e ritrita, ma che descrive appieno l'inizio della guerra che il Como e comuni limitrofi, in collaborazione con l'università di Basilea, ha dichiarato ad una delle peggiori piaghe estive.

Non si tratta dell'esodo estivo o il caldo fuori misura, ma bensì della zanzara tigre. Nella mattinata di ieri, a Palazzo Cernezzi, l'assessore all'Ambiente Bruno Magatti e due ricercatori dell'università di Basilea, Tobias Suter e Begoña Ponsellini, hanno presentato un nuovo progetto di ricerca finalizzato allo studio dell'*Aedes Albopictus*, la zanzara tigre.

Da giugno a ottobre, la città di Como ospiterà ben 28 ovitrappole appositamente poste per permettere lo studio dell'insetto, a partire dalla raccolta di uova e larve.

«L'obiettivo è di stabilire l'assenza, la presenza e l'eventuale abbondanza della popolazione delle zanzare tigre» spiega Tobias Suter, dottorando dell'università di Basilea e ideatore del progetto di ricerca. «Approntare delle trappole serve per acquisire dati sulla biologia di questi insetti e capire quali sono gli insetticidi e i rimedi più efficaci. Al momento della cattura analizzeremo anche il sangue presente nelle zanzare», un modo per capire quali siano le vittime preferite dai fastidiosi insetti che, a differenza delle zanzare comuni, colpiscono soprattutto di giorno.

Le trappole in questione hanno una struttura relativamente semplice. Si tratta di piccoli secchi di plastica con un certo quantitativo d'acqua in cui vengono posti dei listelli di legno. Una volta che la femmina dell'*Albopictus* individua l'ambiente favorevole, dato dall'acqua stagnante, depone le uova a pelo d'acqua sul sostegno di legno.

> **Bruno Magatti**
> **Attenzione ai sottovasi, bastano pochi millimetri d'acqua per le uova.**

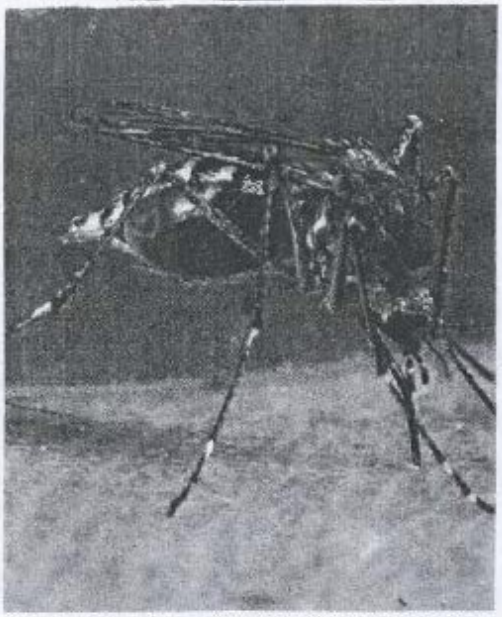
«Ovviamente non vogliamo che la trappola diventi un'incubatrice di esemplari adulti; abbiamo intenzione di inserire nell'acqua un battere per inibire la crescita delle larve – dice Begoña Ponsellini, ricercatrice - Eventualmente, saranno piazzate strisce adesive sul listello di legno per intrappolare le femmine».

I siti in cui approntare le ovitrappole sono stati scelti su tutto il territorio insubrico.

I ricercatori sono riusciti a individuare ben 140 settori adatti, non solo sul territorio italiano ma anche su quello svizzero. Si è pronti a collocare ben 280 ovitrappole posizionate in 140 settori di una vasta area sovranazionale. La parte italiana del progetto, supportato da un'ordinanza comunale, ha avuto inizio ieri con la posatura delle prime due trappole nella zona di torre Gattoni. Altri comuni del territorio hanno patrocinato l'indagine e la posatura delle trappole. Da Brunate ad Olgiate Comasco a Cer-

segue

RASSEGNA STAMPA
COMUNE DI COMO
EVENTI - 3062 ANNO XXXIII
CORRIERE DI COMO
Edizione del 14 GIU. 2012



Un'esemplare di zanzara tigre, caratterizzata dalle striature sul corpo e le zampe

La scheda

Nomenclatura binomia
Aedes Albopictus
Nome volgare
Zanzara tigre
Dove vive
Prevalentemente nelle zone urbane, colonizza secche d'acqua
Caratteristiche principali
Striature bianche su testa, zampe e torace. Aggressiva, diurna e notturna. Non una grande volatrice (in grado di compiere spostamenti pari a 100 metri circa)
Diffusione
Può viaggiare clandestina a bordo di qualsiasi mezzo di trasporto (auto, camion, container)
Contromisure
Evitare accumulo d'acqua stagnante e effettuare la bonifica invernale, con spray insetticidi, di tombini caditoie, solai, ecc., dove le femmine possono svernare

nobbio, tante amministrazioni hanno il desiderio di trovare un modo per ridimensionare la popolazione delle zanzare tigre sul territorio, una specie originaria del sud-est asiatico e insediatasi in Europa dagli anni novanta. Ovviamente è ancora presto per parlare di rimedi definitivi per passare all'offensiva per ridurre la quantità di questi non troppo simpatici animaletti. Tuttavia esistono rimedi per rallentare la diffusione, in attesa di dati e analisi nonché della soluzione adatta.

«Invitiamo tutti i cittadini a fare attenzione alla creazione di fonti di acqua stagnante» ha tenuto a sottolineare l'assessore Bruno Magatti.

«Bastano pochi accorgimenti per evitare di creare dei serbatoi di esemplari e uova di zanzara tigre. Attenzione ai sottovasi, dal momento che bastano anche pochi millimetri d'acqua perché le uova proliferchino e, soprattutto in campagna, i bidoni dell'acqua piovana dovrebbero essere coperti».

Matteo Congregalli

IL GIORNO

COMO I RICERCATORI SVIZZERI STUDIERANNO L'INSETTO CAPACE DI TRASMETTERE 26 MALATTIE

La zanzara tigre nel mirino: pronte 34 trappole in città

PUR LONTANI dalla jungla del Borneo anche in città girano delle tigri, a piede libero e in formato zanzara ma per questo non meno pericolose del grande felino. Lo sanno bene i ricercatori dell'Università di Basilea che ieri hanno avviato in città un monitoraggio dell'Aedes albopictus, detta zanzara tigre, che sembra prediligere Lario e Ceresio. «Si tratta di un insetto molto aggressivo sia di giorno sia di notte – spiegano i ricercatori – che predilige il sangue umano e trasmette ben 26 malattie, alcune delle quali pericolose come la febbre gialla e la dengue. L'insetto è arrivato da noi dal sud-est asiatico, legato, con ogni probabilità, al traffico di copertoni. Le sue uova, infatti, sono molto resistenti e si possono schiudere anche a distanza di 5 mesi». In città sono state collocate 34 trappole, in prossimità delle mura e nei giardini, dall'aspetto di barattoli neri nei quali è immersa acqua e un bastoncino di legno. Lì le zanzare saranno invogliate a deporre le uova e poi studiate.

Ro.Can.

(Servizio riservato agli uffici del Comune di Como)

segue

RASSEGNA STAMPA
COMUNE DI COMO

EVENTI

- 3064

ANNO XXXIII
Edizione del 14 GIU. 2012

La Provincia

Como fa la guerra alla zanzara tigre Con l'aiuto svizzero

L'assessore Magatti tra i ricercatori dell'Università di Basilea

Non sorprendetevi se reperite in qualche angolo della città, in qualche canto nascosto, sotto una siepe o vicino a una fontana, un secchiello pieno d'acqua da cui emerge uno stecco di legno.

Non si tratta di un dimenticato gioco di bimbi, bensì di un serissimo esperimento scientifico presentato ieri mattina dal neo assessore all'ambiente **Bruno Magatti**. Il monitoraggio della zanzara tigre, una delle più aggressive e resistenti, è stato illustrato dal gruppo di lavoro dell'Università svizzera di Basilea, guidato dal dottorando **Tobias Sutter**. Si tratta di collocare delle "trappole", che sono, appunto, questi piccoli secchielli etichettati che vengono riempiti d'acqua (l'ambiente naturale delle zanzare) con uno stecco dove l'insetto deporrà le sue uova.

Prodotti biologici

«Per evitare che le trappole stesse diventino delle colonie di ditteri - hanno precisato i tecnici - viene collocato anche uno speciale prodotto biologico che evita che le uova si schiudano».

Verranno poste in punti casuali della città e del territorio, verranno cambiate ogni quindici giorni e resteranno operative fino al-

RASSEGNA STAMPA
PROVINCIA DI COMO

EVENTI - 5063 ANNO XXXIII

Edizione del 14 GIU. 2012

CORRIERE DI COMO

I consigli dell'entomologo

«Evitate l'accumulo di sacche d'acqua stagnante in giardino»

(m.c.) Dire che prima degli anni Novanta la zanzara tigre non esisteva in Europa suona strano soprattutto se consideriamo la nostra, ormai consolidata, abitudine a trattare con questi aggressivi insetti.

La vicenda della zanzara tigre, caratterizzata dalle striature bianche su zampe, torace e testa, ha qualcosa in comune con alcune delle malattie del millennio passato. Esattamente come la pulce del ratto, vettore della peste nera quella che colpì Firenze nel 1348, per intenderci la zanzara tigre venne importata dall'Asia dai bastimenti commerciali provenienti dall'Oriente.

Leggenda (verificata) vuole che i carichi di copertoni usati arrivati a Genova, poco più di vent'anni fa siano stati un ottimo vettore per questa specie. Le resistenti uova posate nella parte interna dei copertoni furono in grado di resistere alla disidratazione e schiudersi una volta reintrodotte in un ambiente umido e quindi favorevole.

«La particolarità di questo insetto – spiega l'entomologo lariano Mario Colombo - è l'essere sia notturno sia diurno, a differenza della zanzara europea che è attiva soltanto in certi momenti del giorno, nonché la sua particolare e fastidiosa aggressività».

Il paragone tra zanzara e pulce della peste regge anche quando si considera che in zone tropicali la puntura di una zanzara tigre può portare la dengue o la febbre gialla. Nel 2007, nella zona di Ravenna, sono stati registrati un centinaio di casi di Chikungunya, una malattia febbrile non letale, di origine tropicale, trasmessa attraverso al puntura della zanzara tigre. «Quella è stata un' eccezione - precisa Colombo - Con le dovute contromisure la situazione è stata risolta in fretta. In ogni caso è raro che nelle nostre zone la zanzara sia portatrice di malattie».

Mario Colombo
La particolarità di questo insetto è di essere sia notturno sia diurno

Per il territorio lariano, quindi, questo tipo di zanzare rappresenta più un fastidio estivo piuttosto che un vero e proprio rischio. È comunque utile pensare a strategie per arginare la loro diffusione.

«Possiamo intervenire in maniera varia. Introducendo il *Bacillus Thuringensis* (un batterio, ndr) si previene la schiusa delle uova in maniera biologica, cosa preferibile a un intervento con sostanze chimiche che, per quanto calibrato, ha sempre ricadute sull'ambiente», spiega l'entomologo, aggiungendo che un ruolo di contenimento può essere svolto senza troppo sforzo anche dalle singole persone. «Occorre evitare l'accumulo di sacche d'acqua stagnante in giardino, ad esempio, o qualsiasi altro ambiente ideale che faccia da incubatrice alle uova. È l'unica soluzione preventiva. Il celeberrimo filo di rame - conclude Colombo - è più una leggenda metropolitana che qualcosa di scientificamente provato».

segue

RASSEGNA STAMPA
COMUNE DI COMO

EVENTI

ANNO XXXIII
3089
Edizione del 15 GIU. 2012

CORRIERE DELLA SERA

Battaglie d'estate Via al progetto di ricerca. Obiettivo: svelare i punti deboli del fastidioso insetto

Svizzera batte zanzara. Forse

L'Università di Basilea studia a Como la temibile «tigre»

COMO — Italia e Svizzera insieme per dichiarare guerra a uno dei più fastidiosi nemici delle sere d'estate, la zanzara, in particolare quella nota come «tigre», sempre più diffusa nella regione insubrica. Gli esperti dell'università di Basilea hanno avviato la posa di trappole per catturare l'insetto e poi poterlo studiare, naturalmente con l'obiettivo di sconfiggerlo. Segnalate da un adesivo che riporta l'indicazione «Non toccare – Monitoraggio zanzara tigre», le trappole posizionate nel capoluogo sono 34, suddivise in 17 stazioni di osservazione.

Il marchingegno studiato per attirare e catturare la zanzara tigre — nome scientifico *Aedes albopictus*, insetto che può essere lungo dai 2 ai 10 millimetri - è un barattolino contenente acqua e un bastoncino collocato in modo che la femmina vada a deporre le uova. Per evitare che la trappola diventi un comodo punto di riproduzione, nell'acqua viene disciolto un insetticida biologico che impedisce alle uova di schiudersi. Gli studiosi dell'università di Basilea sono arrivati a Como per occuparsi della posa delle trappole, che saranno poi svuotate ogni due settimane fino alla fine di ottobre. «L'obiettivo è di stabilire l'assenza, la presenza e l'eventuale abbondanza della popolazione delle zanzare tigre — spiega Tobias Suter, dell'Università di Basilea e ideatore del progetto di ricerca —. Posizionare le trappole serve per acquisire dati sulla biologia di questi insetti e capire quali sono gli insetticidi e i rimedi più efficaci. Al momento della cattura analizzeremo anche il sangue presente nelle zanzare». Importata in Italia dal Sudest asiatico nei primi anni 90, e rapidamente diffusasi a spese della specie autoctona (per questo viene considerata una delle 100 specie aliene più pericolose al mondo), secondo gli esperti soprattutto tramite il commercio di copertoni, la zanzara tigre non è solo fastidiosa ma anche potenzialmente pericolosa.

«La zanzara tigre — spiega ancora Suter — è un insetto attivo anche di giorno, molto aggressivo, che preferisce il sangue umano e che trasmette ben 26 malattie, alcune delle quali pericolose come la febbre gialla e la dengue. Le uova sono molto resistenti e si possono schiudere anche a distanza di 5 mesi e questo ha permesso che arrivassero in Italia». «Sulla base di questo studio — precisa l'assessore all'Ambiente del Comune di Como, Bruno Magatti — sarà possibile capire e misurare la presenza di questo insetto e quindi valutare i necessari interventi. Più informazioni scientifiche si hanno a disposizione e più efficacemente potremo combattere la zanzara tigre».

Anna Campaniello

Specie aliena

34 Le «trappole» collocate nel territorio comunale di Como per lo studio della zanzara tigre

Diffusione
La zanzara tigre (*Aedes Albopictus*), originaria del sudest asiatico, si è diffusa in Italia all'inizio degli ottni anni 90

Le trappole
Nella foto grande, i due ricercatori dell'Università di Basilea con una delle trappole anti-zanzara

Accordo regionale

E il pesce-siluro finisce al mercato

MILANO — Sono diventati un flagello, però hanno un mercato tutt'altro che trascurabile. I pesci siluro, carassio e gardon, arrivati dall'Europa centro-orientale, hanno infestato laghi e fiumi lombardi, predando le specie locali; però il mercato ittico di Milano li importa perché sono richiesti, per esempio, dalla comunità rumena in Italia. Per questo domani l'assessore regionale all'agricoltura Giulio De Capitani firmerà un accordo per commercializzarli: anche i mercati est-europei potrebbero essere interessati.

8.2.2 *Article of "Neue Zürcher Zeitung" (NZZ)*

NZZ.CH — Neue Zürcher Zeitung — ÜBERSICHT

20.11.2013, 10:30 Uhr

Gestreifte Eindringlinge

Auf Tigermückenjagd in der Schweiz

Martin Amrein 20.11.2013, 10:30 Uhr

Im Labor der kantonalen Mückenarbeitsgruppe in Lugano züchten Biologen «Schweizer» Tigermücken. *(Bild: Roland Schmid)*

Europa bekommt es mit der Asiatischen Tigermücke zu tun. Zeit zu erforschen, wie gefährlich sie ist, welche Gifte gegen sie wirken – und ob sie es bereits in die Deutschschweiz geschafft hat.

Zielstrebig stapft Tobias Suter auf einen Pfosten am Ende der Pferdeweide zu. Er bückt sich und hebt einen kleinen, schwarzen Plasticbecher auf, der mit Wasser gefüllt ist. Ein zwanzig Zentimeter langes Holzstück ragt daraus hervor. Es ist voller dunkler Punkte: «Alles Mückeneier. Wahrscheinlich Aedes geniculatus», sagt der junge Forscher nach einem prüfenden Blick. Diesmal stammen die Eier nicht von Aedes albopictus, der invasiven Asiatischen Tigermücke, die mit der einfachen, aber äusserst wirksamen Falle nachgewiesen werden soll, sondern von einer heimischen Spezies. Die Mücken halten den dunklen Becher für einen idealen Brutplatz und legen ihre Eier auf das darin liegende Holzstäbchen ab. Die endgültige Artbestimmung erfolgt später im Labor.

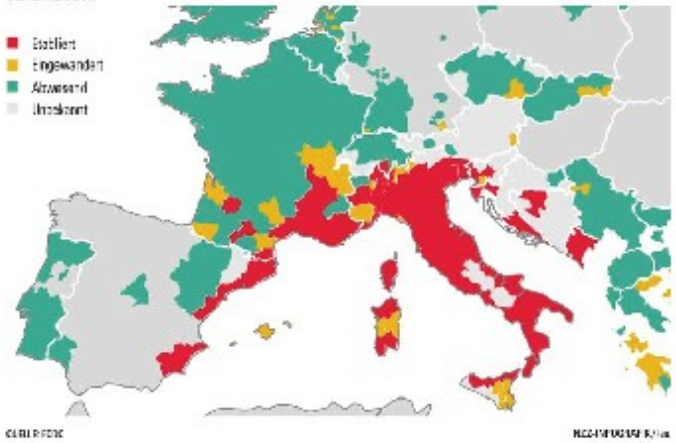
Autoreifen als Brutplätze

Wir befinden uns am Rand eines kleinen Waldstücks in der Nähe von Mendrisio, im Tessin. Suter, der die hier ausgelegten Fallen kontrolliert, ist Biologe und doktoriert am Schweizerischen Tropen- und Public-Health-Institut (Swiss TPH) in Basel. Während der Feldsaison, die noch bis Ende November dauert, wohnt er in Roveredo. Im Rahmen seines Forschungsprojektes untersucht er unter anderem, wie weit sich die Asiatische Tigermücke südlich der Alpen verbreitet hat und wie anfällig sie auf bestimmte Insektizide ist.

Die Asiatische Tigermücke, potenzielle Überträgerin gefährlicher Viren, stammt ursprünglich aus Südostasien. Natürlicherweise legen die Mücken ihre Eier in Baumhöhlen ab. Aber auch in kleinen Pfützen, in Autoreifen oder Blumengefässen finden sie ideale Brutplätze. Im Zuge der Globalisierung haben sie sich so über

Verbreitung der Asiatischen Tigermücke in Mittel- und Südeuropa

Stand März 2013



Transportrouten weltweit ausgebreitet. Nachdem sich die Insekten bereits in den neunziger Jahren in Norditalien niedergelassen hatten, wanderten sie weiter nordwärts. Tessiner Forschern der kantonalen Mückenarbeitsgruppe gelang es 2003 erstmals, Asiatische Tigermücken in der Südschweiz nachzuweisen. Mittlerweile haben sich die Blutsauger im Kanton etabliert; es gibt verschiedene Populationen sich fortpflanzender Tiere.

30 Standorte pro Tag

Um die Mücken aufzuspüren, hat Suter an 140 zufällig ausgewählten Standorten im Tessin und in Norditalien je zwei Fallen aufgestellt. Ungefähr 30 Standorte kontrolliert er pro Tag – ein Knochenjob. Obwohl er seine Tour jeweils früh beginnt, ist er mit seinem Kleinwagen oft bis in die Abendstunden auf holprigen Landstrassen unterwegs. Ist er einmal nicht im Feld, verbringt Suter die Tage im Labor der Mückenarbeitsgruppe in Lugano. Dort stapeln sich an seinem Arbeitsplatz mit Mückeneiern übersäte Holzstäbchen. Nachdem er mit dem Mikroskop die Art bestimmt habe, berichtet Suter, zähle er die Eier einzeln ab. Einmal habe er auf einem einzelnen Holzstück 2200 Eier gefunden. Anhand der so gewonnenen Daten kann er später die saisonale und räumliche Verteilung der Mücken abschätzen.

Im selben Grossraumbüro wie Suter arbeitet die Biologin Eleonora Flacio. In der Mückenarbeitsgruppe koordiniert sie die Bekämpfung der Tigermücken im Tessin. Zwei- oder dreimal pro Jahr bietet sie den Zivilschutz auf, um besonders mückenreiche Gebiete mit dem Insektizid Diflubenzuron zu behandeln. Dabei sei es wichtig, die richtige Dosierung zu beachten, weil das Gift, wenn es in Gewässer gelange, auch Fische gefährden könne, sagt Flacio. Einfacher sei die Handhabung des Bti-Toxins, das keine Nebeneffekte habe. Dieses Insektizid kann die Bevölkerung selber gegen die Mücken einsetzen, etwa in Form kleiner Körner, die in Gullys gekippt werden. Entscheidend ist laut Flacio aber auch, die Menschen für die Mückenproblematik zu sensibilisieren. Achte man darauf, den Mücken keine Brutplätze in Blumentopfuntersätzen oder Regentonnen zu bieten, sei schon viel getan.

Biologe Tobias Suter sammelt Tigermücken-Eier mit gezogenen Fallen. (Roland Schmid)

Nicht von ungefähr ist den Behörden viel daran gelegen, die Tigermücken in Schach zu halten, sind die Insekten doch fähig, über 20 verschiedene Viren zu übertragen. Dazu gehören das Chikungunya- und das Denguevirus. Das sind tropische Erreger, die in der Schweiz nicht heimisch sind, aber von Touristen eingeschleppt werden können. Geschehen ist dies 2007 im norditalienischen Ravenna. Bei einer lokalen Epidemie erkrankten damals rund 200 Personen am Chikungunya-Fieber.

Ähnliches könne theoretisch auch in der Schweiz passieren, die Wahrscheinlichkeit dafür sei jedoch viel geringer, weil die Populationsdichten der Tigermücken hier ungleich kleiner seien als in Italien, sagt Pie Müller vom Swiss TPH, der Suters Forschungsarbeit sowie das gesamtschweizerische Projekt zur Überwachung der Tigermücke leitet (siehe Kasten). Sicherheitshalber untersuche Suter schon jetzt, ob Tigermücken auch unter den klimatischen Bedingungen der Nordschweiz imstande sind, Dengueviren zu übertragen. Denn es sei damit zu rechnen, dass sich die äusserst anpassungsfähigen Tiere zukünftig auch nördlich der Alpen etablieren, derzeit kämen dafür aber wahrscheinlich nur die wärmeren, städtischen Regionen Genf und Basel infrage.

Nicht konsequent bekämpft

Im Tessin greifen derweil die Kontrollmassnahmen, die seit zehn Jahren im Gang sind. Seine Daten zeigten, dass auf der Schweizer Seite der Grenze viel weniger Tigermücken vorhanden seien als auf der italienischen, wo die Tiere nicht konsequent bekämpft würden, sagt Suter.

Um lebende Mücken zu fangen, fahren wir an diesem Nachmittag denn auch über die Grenze: Die Falle, die Suter auf einer Wiese südlich von Bizzarone aufstellt, gleicht einem zylindrischen Lampion. In ihrem Innern verbirgt sich ein muffig riechendes Lockmittel, ein kleiner Ventilator saugt heranschwirrende Mücken an. Später wird der Forscher das Blutmahl von Tigermücken, die er an verschiedenen Orten gefangen hat, untersuchen, um ihre bevorzugten Wirte festzustellen. Ziemlich sicher steht menschliches Blut weit oben auf ihrer Speisekarte. Jedenfalls kommen die Mücken in besiedelten Gebieten häufiger vor als in Wäldern.

In den Wintermonaten wird Suter dann selber Tigermücken züchten. Dazu verwendet er Tiere, die aus den gesammelten Eiern geschlüpft sind. Ziel ist es

herauszufinden, wie die «Schweizer» Tigermücken auf verschiedene Insektizide reagieren und ob sie allenfalls schon Resistenzen entwickelt haben. Er werde versuchen, die Mücken mit Schweineblut zu füttern, sagt Suter. Klappe das nicht, müsse halt sein eigener Arm herhalten, um den Hunger der Blutsauger zu stillen. Der junge Wissenschafter erledigt seine Arbeit wirklich mit ganzem Körpereinsatz. Schon im nächsten Frühling will er all seine Analysen abgeschlossen haben.

Erste Exemplare nördlich der Alpen?

mna. - Ausser im Tessin gibt es in der Schweiz bis jetzt noch keinen wissenschaftlichen Nachweis der Asiatischen Tigermücke. Zwar geisterten in den vergangenen Jahren schon Meldungen durch die Medien, dass die Mücke in der Deutschschweiz beobachtet worden sei. Laut Pie Müller vom Swiss TPH handelte es sich dabei aber wahrscheinlich um Verwechslungen mit der hier weitverbreiteten Japanischen Buschmücke (Aedes japonicus), die der Tigermücke stark ähnelt. Nur ein geschultes Auge kann die Arten unterscheiden.

Deshalb gilt die Asiatische Tigermücke in der Deutschschweiz offiziell nicht als «eingewandert» (einzelne Tiere vorhanden), sondern als «abwesend». Das könnte sich aber bald ändern. Im Auftrag des Bundesamtes für Umwelt (Bafu) hat das Swiss TPH in den vergangenen Monaten eine schweizweite Kontrolle durchgeführt. Mit Fallen, wie sie Tobias Suter verwendet, stellten Forscher der Mücke entlang der Hauptverkehrsachsen nach: an Autobahnen, Bahnhöfen, Flugplätzen und Häfen.

In den kommenden Tagen wird das Bafu über die Ergebnisse berichten. Weil im letzten Jahr und auch wieder in diesem Herbst in Süddeutschland Tigermücken nachgewiesen wurden und auch im französischen Rhonetal Tigermücken vorkommen, wäre es verwunderlich, wenn nicht auch in der Deutsch- oder der Westschweiz einzelne Tiere anzutreffen wären. Zumal Experten davon ausgehen, dass die Mücken über die Schweiz mit dem Güterverkehr nach Deutschland gelangt sind.

MEHR ZUM THEMA

Die Tigermücke fliegt nordwärts
26.4.2012, 14:13 Uhr

Stechmücken
Wider die Plagegeister
26.7.2012, 12:05 Uhr

Warmes Frühlingswetter
Eldorado für Insekten
28.3.2014, 09:47 Uhr

Gefahr für Reisende
Dengue-Fieber breitet sich rasant aus
1.4.2014, 23:31 Uhr

8.2.3 *Programme of RSI on A. albopictus in Ticino*

Tigri alla conquista del pianeta

Di Enrico Pasotti, trasmesso giovedì 19 settembre 2013 - Temi: Ambiente / Salute / Scienze - Regioni: Esteri / Svizzera italiana

Non conoscete la zanzara tigre che svolazza anche da noi? Meglio così! Punge di giorno, ripetutamente, anche attraverso i vestiti. Non fa distinzione tra uomini e animali, punge persino i rettili, nessuno si salva. Ma c'è di più, potrebbe diffondere malattie temibili come la Dengue o la Chikungunya. Falò ha seguito la caccia alle tigri del Gruppo lavoro zanzare per conoscerla e capire come la si combatte. La zanzara tigre è stata registrata per la prima volta a Lugano nel 2004, ma negli ultimi anni si assiste a una vera e propria esplosione della presenza della zanzara nel Sottoceneri.

RSI - Falò - Stampato il 13 marzo 2015
http://www.rsi.ch/falo/index.cfm?scheda=23723

8.2.4 *Programme of 3sat: "Berg & Geist"*

3sat

3sat.de Homepage > Sendungen A-Z > Infoseite

Einmal im Monat, um 22.25 Uhr

Berg und Geist

Berg und Geist: Silvia Tschui

«Nur durch die Kunst kann sich die Menschheit vom Tierischen retten», sagt Silvia Tschui.

Porträtreihe

Berg und Geist: Tobias Suter

Mit dem Tigermückenexperten Tobias Suter auf dem Monte Generoso

Der junge Schweizer Biologe Tobias Suter ist in Tansania geboren, wo sein Vater für das Schweizerische Tropeninstitut (Swiss TPH) tätig war. Schon immer ein Naturfreund folgte Tobias schliesslich den Fusstapfen seines Vaters und arbeitet heute in Basel für dasselbe Institut. Sein Spezialgebiet sind die sogenannten Tigermücken, potenzielle Überträger von gefährlichen Viren wie das Chikungunya- und das Dengue-Virus, die sich derzeit vor allem südlich der Alpen ausbreiten und jeden Sommer für schmerzhafte Stiche sorgen.

In der Schule interessierte sich Tobias Suter einzig für Biologie: Er war nämlich viel lieber draussen in der Natur als im Klassenzimmer. Auch heute kann er sich nicht vorstellen, nur im Labor tätig zu sein, so spannend diese Arbeit auch ist. Aus diesem Grund suchte er sich ein Gebiet mit möglichst viel Feldarbeit, und bewarb sich für ein Projekt zur Überwachung der Tigermücken im Tessin und in Norditalien. Seine umfangreiche Arbeit zeigt, dass trotz der erfolgreichen Bekämpfung im Tessin eine Ausbreitung der gefährlichen Tigermücke nördlich der Alpen nur eine Frage der Zeit ist.

Für «Berg und Geist» steigt der Schweizer Biologe auf den Monte Generoso im Tessin und zeigt, wie er in Asthöhlen Mückenlarven jagt, um sie dann in seinem Labor auszubrüten und schliesslich einmal in der Woche, am eigenen Arm, bis zu 600 Mücken mit Blut vollsaugen zu lassen.

Sendedaten

Montag, 25. August 2014
23.10 Uhr

August 2014 / SRF, MM

8.3 GLZ Flyer

Helfen Sie uns die Tigermücke einzudämmen!

Wieso bekämpft man die Tigermücke?

- Um die Lebensqualität zu schützen: sie ist sehr aggressiv, sticht mehrmals pro Blutmahl, ist tagesaktiv und besiedelt urbane Lebensräume
- Um Krankheitsübertragungen zu vermeiden: die Tigermücke kann potentieller träger mehrerer ernsten Krankheiten sein

Was machen die Gemeinden und der Gruppo cantonale di lavoro zanzare (GLZ)?

- Seit dem Jahr 2000 betreibt der GLZ ein präventives Überwachungsnetz im Kanton Tessin
- Die Gemeinden arbeiten sowohl bei der Überwachung wie bei der Bekämpfung auf öffentlichem Grund eng mit dem GLZ zusammen

Wie kann ich sie erkennen ?

- Ihr Aussehen und Grösse (ca.0.5 - 1 cm) ähneln einer gemeinen Stechmücke, sie ist aber deutlich schwarz mit weissen streifen (tatsächliche Grösse: siehe Bild nebenan)

Wie entwickelt sie sich ?

- Jede weibliche Mücke legt im Laufe ihres Lebens, welches ca. 1 Monat dauert, hunderte Eier, die sich in einer Woche zu neuen Adulten entwickeln, welche ihrerseits ebenso viele Eier legen
- Die Eier werden vorwiegend in kleine Wasseransammlungen gelegt: die Menge eines Bechers genügt!
- Die Tigermücke klebt ihre Eier oberhalb des Wasserpegels an die Wand des Behälters, diese schlüpfen nicht simultan, sind über mehrere Monate trockenresistent, können den Winter überdauern und im Frühling wieder schlüpfen, wenn sie erneut mit Wasser überschwemmt werden

Wie verbreitet sie sich ?

- Sie fliegt ziemlich schlecht (legt nur kurze Distanzen zurück, unter 100 m), sie vermehrt sich also in der Nähe des Beobachtungsortes
- Sie verbreitet sich über längere Distanzen als Schwarzfahrerin mittels Fahrzeugverkehr (PKW, LKW, Container, usw.)

Was kann jeder Einzelne dagegen tun?

- Die Zusammenarbeit der Bevölkerung ist entscheidend!
- Um deren Entwicklung zu unterbrechen, muss man jegliches stehendes Wasser vermeiden: um unsere Behausungen befinden sich zahlreiche Behälter, welche sich durch Regen oder Bewässerung mit Wasser füllen können und zu potentielle Brutstätten werden; in fliessendem Wasser (Fliessgewässer, Springbrunnen, usw.) können die Larven nicht überleben
- Sollten Sie einen Verdacht haben eine Tigermücke gesichtet zu haben, kontaktieren Sie bitte den GLZ!

Für weitere Infos und/oder Signalisierungen:
http://www.ti.ch/zanzare/

Gruppo cantonale di Lavoro Zanzare (GLZ)
Antenna Istituto di Microbiologia Applicata,
via al Castello,
6952 Canobbio

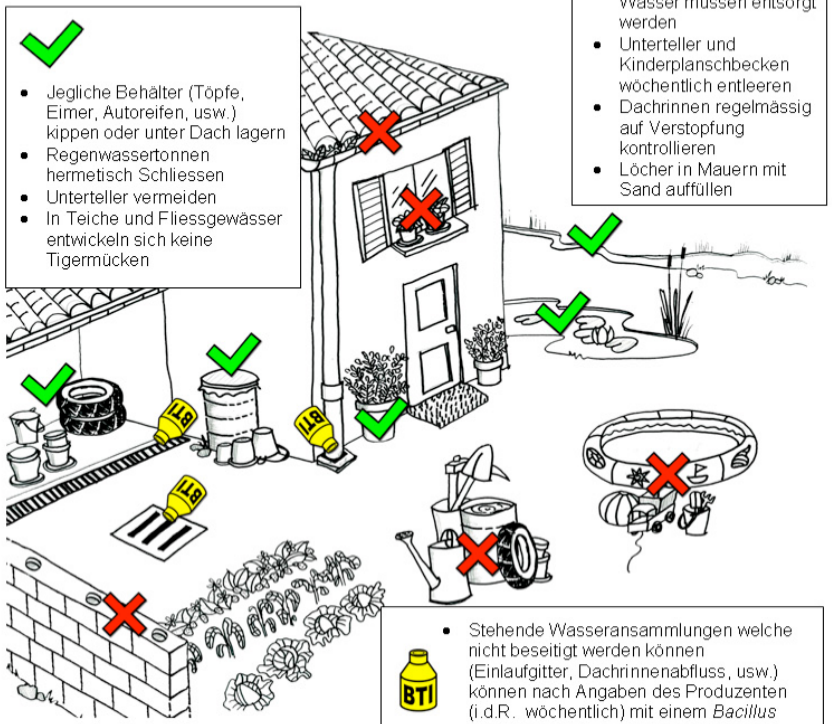
telefono 091 935 00 46
e-mail: dss-us.zanzaratigre@ti.ch

Wie kann man sie bekämpfen ?

- Von April bis November sollten jegliche Behälter welche sich mit Wasser füllen könnten vermieden werden: kippt sie um oder lagert sie unter Dach
- Unterteller, Kinderplanschbecken, Tränken, usw. müssen mindesten einmal in der Woche austrocknen
- Regenwassertonnen sollten hermetisch abgeschlossen werden und nur bei Regen geöffnet werden
- In Teichen und Fliessgewässer kann sich die Tigermücke nicht entwickeln: Fische und Amphibien fressen die Larven
- Die Tigermückenlarven können sich auch in Felsspalten oder Löchern wo Wasser steht entwickeln: füllt diese mit Sand

- Jegliche Behälter (Töpfe, Eimer, Autoreifen, usw.) kippen oder unter Dach lagern
- Regenwassertonnen hermetisch Schliessen
- Unterteller vermeiden
- In Teiche und Fliessgewässer entwickeln sich keine Tigermücken

- Behälter mit stehendem Wasser müssen entsorgt werden
- Unterteller und Kinderplanschbecken wöchentlich entleeren
- Dachrinnen regelmässig auf Verstopfung kontrollieren
- Löcher in Mauern mit Sand auffüllen



- Stehende Wasseransammlungen welche nicht beseitigt werden können (Einlaufgitter, Dachrinnenabfluss, usw.) können nach Angaben des Produzenten (i.d.R. wöchentlich) mit einem *Bacillus thuringiensis israeliensis* (BTI) haltigen Larvizid behandelt werden

8.4 Supplementary information

Table S1. Number of invalid ovitraps. N indicates the number of invalid ovitraps (trap damaged or missing, slat missing).

Year	Run	Country	Stratum	N
2013	1	CH	urban	9
2013	2	CH	urban	9
2013	3	CH	urban	7
2012	4	CH	urban	1
2013	4	CH	urban	28
2012	5	CH	urban	1
2013	5	CH	urban	2
2012	6	CH	urban	4
2013	6	CH	urban	3
2012	7	CH	urban	6
2013	7	CH	urban	1
2012	8	CH	urban	8
2013	8	CH	urban	1
2013	9	CH	urban	6
2012	10	CH	urban	1
2013	10	CH	urban	8
2013	11	CH	urban	2
2013	12	CH	urban	3
2013	13	CH	urban	7
2013	1	IT	urban	11
2013	2	IT	urban	6
2012	3	IT	urban	1
2013	3	IT	urban	9
2012	4	IT	urban	1
2013	4	IT	urban	10
2012	5	IT	urban	2
2013	5	IT	urban	4
2012	6	IT	urban	3
2013	6	IT	urban	3
2012	7	IT	urban	2
2013	7	IT	urban	3
2012	8	IT	urban	4
2013	8	IT	urban	3
2013	9	IT	urban	14
2012	10	IT	urban	1
2013	10	IT	urban	3
2013	12	IT	urban	7
2013	13	IT	urban	2
2013	1	CH	sylvatic	5
2013	2	CH	sylvatic	4
2012	3	CH	sylvatic	1

2013	3	CH	sylvatic	4
2012	4	CH	sylvatic	1
2013	4	CH	sylvatic	19
2012	5	CH	sylvatic	1
2012	6	CH	sylvatic	2
2013	6	CH	sylvatic	1
2013	7	CH	sylvatic	1
2012	8	CH	sylvatic	4
2013	8	CH	sylvatic	3
2013	9	CH	sylvatic	4
2013	10	CH	sylvatic	6
2013	11	CH	sylvatic	4
2013	12	CH	sylvatic	4
2013	13	CH	sylvatic	2
2013	1	IT	sylvatic	8
2013	2	IT	sylvatic	8
2013	3	IT	sylvatic	9
2012	4	IT	sylvatic	1
2013	4	IT	sylvatic	13
2012	5	IT	sylvatic	1
2013	5	IT	sylvatic	3
2013	6	IT	sylvatic	2
2012	7	IT	sylvatic	3
2013	7	IT	sylvatic	3
2012	8	IT	sylvatic	5
2013	8	IT	sylvatic	4
2013	9	IT	sylvatic	12
2012	10	IT	sylvatic	1
2013	10	IT	sylvatic	5
2013	12	IT	sylvatic	15
2013	13	IT	sylvatic	2

Chapter 9

Curriculum vitae

TOBIAS SUTER

Müllheimerstrasse 138
4057 Basel
Switzerland

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Mobile +41 79 825 57 06

An expert in the field of vector biology and control, with a creative, problem-solving mind and excellent team work and communication skills.

Professional Experience

University of Basel, Swiss Tropical and Public Health Institute
Doctoral Researcher **04.2011 to present**

- Outlining and planning research project objectives and scheduling activities, reviewing outcomes and results, and identifying consequent resource needs.
- Developing research partnerships with three external institutes in Switzerland, Italy, and Brazil.
- Running an independent monitoring project for the Asian tiger mosquito *Aedes albopictus* in the Swiss-Italian border region.
- Largely involved in the national surveillance programme for invasive mosquito species in Switzerland.
- Rearing field-caught mosquito populations in the laboratory and measuring their insecticide susceptibility status.
- Working in different countries (one year in Italy and four months in Brazil).
- Performing vector competence studies with field-collected mosquito populations.
- Evaluating methods for the collection of blood-fed mosquito females.
- Working with colleagues from diverse backgrounds.
- Teaching lessons in courses (“Allgemeiner Tropenkurs”, 8 hours) with 20 participants.
- Interacting with politicians, newspapers and television, including meetings, interviews and a portrait.
- Using four different working languages (DE/EN/FR/IT)

Gymnasium Kirschgarten, Basel
Supply Teacher ***11.2015***

- Teaching basic biology to a class of 15 fourteen year old students (8 hours)
- Preparing lectures and practical work

Securitas AG, Basel
Security worker **01.2007 to 01.2010**

- Event services, security services, traffic services, intervention and control services.
- Insights in several companies (i.e. chemical industry, pharmaceutical industry, food production, harbours)
- Working trilingually (DE/FR/EN)
- Working independently in uncomfortable environments, solving practical problems on the spot

Educational Qualifications

- PhD in Epidemiology and Public Health, Swiss TPH **expected February 2016**
- Master in Epidemiology and Infection Biology, University of Basel **2011**
  - Investigating mosquito species richness in the area of Basel and the vicinity of the Airport Basel-Mulhouse (CH/FR).
  - Identifying and mapping mosquito breeding sites.
  - Collecting mosquito larvae with standard techniques.
  - Identifying mosquitoes to species level (larvae and adults)
- Bachelor in Biology, University of Basel **2009**

Professional Training

Fundraising and Proposal Writing, Basel, Switzerland **2015**
Project Management for Research, Basel, Switzerland **2015**
IVCC-AvecNet QC Workshop, Cotonou, Benin **2015**
GIS in Public Health, Basel, Switzerland **2014**

Languages

German (native speaker)
English (working language C1/C2)
French (good knowledge B2)
Italian (basic knowledge B1)

Computer Skills

MS Office (Mac and PC), ArcGIS, Stata

Memberships

- Swiss Vector Entomology Group (SVEG)
- European Society for Vector Ecology (EMCA)

Scientific Journal Articles

- Tobias Suter, Eleonora Flacio, Begoña Feijoó Fariña, Lukas Engeler, Mauro Tonolla, and Pie Müller: First report of the invasive mosquito species *Aedes koreicus* in the Swiss-Italian border region. Parasites & Vectors. 2015; 8: 402.
- Suter et al.: The impact of surveillance and control on the *Aedes albopictus* population in the Swiss-Italian border region: Comparison of an intervention vs. a non-intervention area. Submitted to PLoS NTD. 2015.

Reference

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