

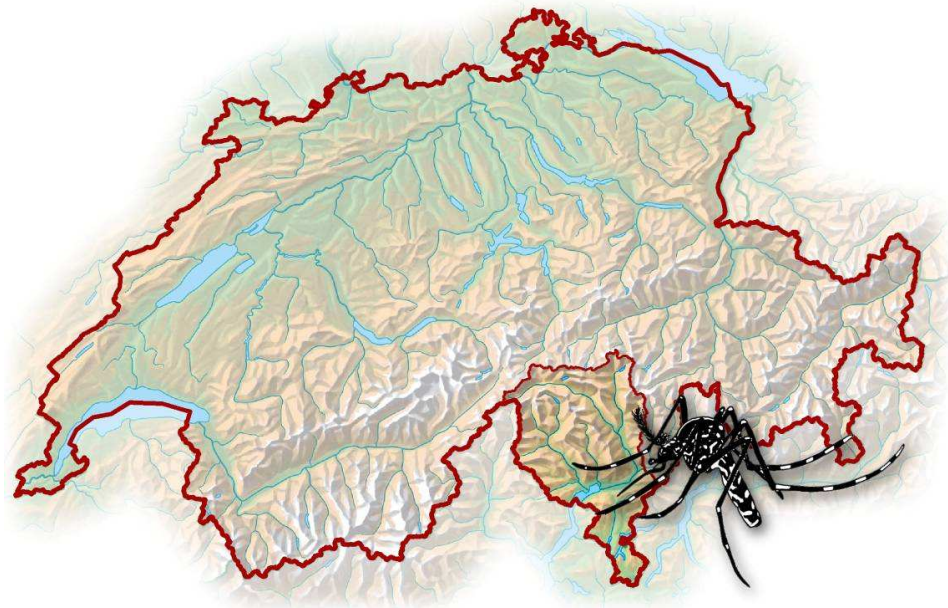
Long term survey and control strategies of mosquitoes in southern Switzerland with focus on the invasive exotic species *Aedes albopictus*

THESIS

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BY

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COVER PAGE PICTURE: Lukas Engeler, Francesco Pace and Eleonora Flacio on Swisstopo map.

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**“Long term survey and control strategies of
mosquitoes in southern Switzerland with focus
on the invasive exotic species
Aedes albopictus”**

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Neuchâtel, le 14 avril 2016

Le Doyen, Prof. B. Colbois



“If you think you are too small to be effective, you have never been in bed with a mosquito.”

Dalai Lama

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1 ABSTRACT

1. ABSTRACT

Until 2000 in Canton Ticino, a Swiss region located South of the Alps, the knowledge on mosquito distribution was scarce. Some faunistic investigations related to malaria occurrence in the area were lead until the beginning of last century and others started at the end of that century to support control measures against wetland mosquitoes causing nuisance in residence areas close to lake Maggiore. A general evaluation on mosquito species was needed to assess the biodiversity in all main natural areas, and on the origin of nuisance to improve the control measures on mosquitoes. In addition the knowledge of the location and the densities of the different mosquito species would give an important tool to evaluate the risk of transmission of mosquito-borne pathogens, such as West Nile virus. Not only European native species needed investigations but also exotic species, like *Aedes albopictus*, which was rapidly spreading in the nearby Italian urban settlements since the 90's. These exotic species are known as extremely bothersome and as potential vectors of several pathogens that could affect human and animal populations on the European territory.

With a combination of several sampling techniques all main natural areas from Ticino were screened. New mosquito species for Ticino and/or Switzerland were discovered: *Aedes caspius*, *Ae. cataphylla*, *Culex martini*, *Cx. modestus*, *Coquillettidia buxtoni* and *Cq. richiardii*. Control measures already undertaken showed their efficacy and risk of transmission of mosquito-borne pathogens was evaluated as very low.

A surveillance system on *Ae. albopictus* was set up in 2000. This permitted to follow up the mosquito activity and spread from its first discovery in 2003 in the South of Canton Ticino, and to adopt coordinated control measures involving scientists, municipalities and residents. This surveillance has developed through different strategies according to *Ae. albopictus* presence in the territory. The public participation in measures to limit the mosquito presence represents a key tool of the surveillance strategy. This permitted to keep the mosquito presence at a reasonable level. Although the continuous spread in the region could not be avoided no local transmission of exotic diseases was recorded so far.

Our data showed that the mosquito was introduced via the intense road traffic and then spread thanks to local traffic. Weather conditions allowed its establishment in the South. In the North and at altitudes above 400 m its presence is sporadic probably because of lower temperature and road traffic.

The *Ae. albopictus* survey permitted to discover additional exotic species new in Canton Ticino and / or Switzerland, which were probably also introduced via road traffic: *Ae. cretinus*, *Ae. koreicus* and *Ae. japonicus*.

Key words: *Aedes albopictus*, exotic mosquito, invasive mosquito, surveillance, control measures, spread, *Aedes caspius*, *Aedes cataphylla*, *Culex martinii*, *Culex modestus*, *Coquilletidia. buxtoni*, *Coquilletidia richiardii*, *Aedes japonicus*, *Aedes koreicus*

RÉSUMÉ

Les connaissances sur les moustiques présents au Tessin, un canton suisse situé au Sud des Alpes, étaient faibles jusqu'aux années 2000. Au début du siècle dernier, des recherches faunistiques ont été menées dans le cadre de la malaria et, plus tard, d'autres recherches ont été entreprises pour lutter contre les moustiques des marais qui gênaient les populations autour du lac Maggiore. Cependant, les connaissances sur la diversité des moustiques dans les principales zones humides ainsi que sur leur lieu d'origine et leur densité restaient insuffisantes pour lutter efficacement contre leur nuisance et les risques de transmission de pathogènes, comme par exemple le virus West Nile.

Dans ce travail, nous avons approfondi les connaissances sur les moustiques indigènes ainsi que sur les moustiques exotiques, comme *Aedes albopictus*, le moustique tigre, qui se répandait dans les zones urbaines italiennes depuis les années 90. Les principales zones humides du Canton du Tessin ont été investiguées en utilisant différentes techniques de récolte. Ainsi, de nouvelles espèces de moustiques ont été découvertes: *Ae. caspius*, *Ae. cataphylla*, *Culex martini*, *Cx. modestus*, *Coquilletidia buxtoni* and *Cq. richiardii*. Les traitements de lutte qui étaient déjà utilisés sont apparus comme efficaces pour lutter

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contre les moustiques nuisibles pour la population. En 2000 un système de surveillance d'*Ae. albopictus* a été mis en place. Ceci a permis de suivre l'activité et la diffusion du moustique depuis sa découverte en 2003 dans le Sud du canton et d'adopter des mesures coordonnées entre scientifiques, mairies et population. Ce système de surveillance s'est développé au cours du temps. Différentes stratégies ont été appliquées en fonction de la distribution du moustique sur le territoire. La participation de la population aux mesures de surveillance a été considérée comme fondamentale. Nous avons donc mis en place un système d'information ciblé pour la population lui permettant de participer activement à la surveillance. La présence du moustique a pu ainsi être maintenue à des niveaux raisonnables mais sa continuelle avancée dans le territoire n'a cependant pas pu être parée. Nous avons pu établir que le moustique s'est introduit au Tessin via le trafic autoroutier, puis s'est étendu à l'intérieur du canton grâce au trafic local. Le moustique est aujourd'hui bien établi dans le sud où les conditions météorologiques lui sont favorables. Au nord, et à plus de 400 m d'altitude, il n'apparaît que sporadiquement probablement en raison de conditions météorologiques moins favorables et d'un trafic routier moins intense.

En surveillant *Ae. albopictus* nous avons découvert d'autres espèces exotiques non encore décrites au Tessin ou en Suisse: *Ae. cretinus*, *Ae. koreicus* et *Ae. japonicus*, qui ont emprunté les mêmes voies d'introduction qu'*Ae. albopictus*.

Actuellement, le risque de transmission de maladies liées aux différentes espèces de moustiques répertoriées dans le canton peut être considéré comme faible et à ce jour, aucun cas de transmission locale de maladies n'a été relevé.

Mots clés: *Aedes albopictus*, moustique exotique, moustique invasive, surveillance, mesures de contrôle, dispersion, *Aedes caspius*, *Aedes cataphylla*, *Culex martinii*, *Culex modestus*, *Coquilletidia. buxtoni*, *Coquilletidia richiardii*, *Aedes japonicus*, *Aedes koreicus*

2 INTRODUCTION

2. INTRODUCTION

The mosquitoes (Culicidae Meigen, 1818) belong to a family of Insecta of the order of Diptera (Nematocera). The mosquitoes are included in one of the most primitive families of Diptera, the Culicidae, whose origin is dated in the Mesozoic era and the most antique fossil dates back to the Jurassic inferior period (175.6-199.6 millions of years ago) (Edwards 1923). This family counts over 3,500 species in the world, around 100 in Europe (Ramsdale and Snow 1999) and 35 are reported in the Swiss checklist (Briegel 1998, Merz et al. 2006). Mosquitoes can be found all over the world except at places that are permanently frozen. Three quarters of the mosquito species are concentrated in warm and humid zones of subtropics and tropics, where there are favourable conditions for their development and survival. In the arctic zone the climate permits the development of a dozen of species, but they develop in mass once when the snow melts. Nowadays this regional distribution of species is changing because of global movement of persons and goods and climate changes, which permitted to exotic species to arrive and establish elsewhere, like for example in Europe (Medlock et al. 2012, 2015).

The notoriety of the mosquitoes is mostly related to their bites. In fact the females of many species have mouthparts adapted for piercing the skin of vertebrate animals and sucking their blood. This ectoparasite activity permits the gravid mosquito to obtain protein resources for egg development. Moreover this activity allows the transmission to the host of several pathogens that co-evolved with the mosquitoes, and which are responsible for the so-called mosquito-borne diseases. In fact some species of mosquitoes can act as vectors of several microorganisms to animals and/or to humans. It is estimated that about half of the world population lives under the threat of diseases transmitted by mosquitoes, close to 300 millions suffer from the diseases and about one million die (WHO 2009, "WHO | World Malaria Report 2014" 2015), which makes the mosquito the most dangerous animal to humans. Among these diseases the most well known are malaria, dengue, yellow fever, chikungunya, filariasis, etc. The recent spread and the establishment of exotic mosquitoes, as for example the tiger mosquito *Aedes albopictus* (*Stegomyia albopicta*), in parallel with the introduction of exotic disease, mainly from travellers

returning from holidays, creates concern in Europe for the possibility of epidemic of diseases confined so far in tropical and subtropical regions.

2.1 SYSTEMATIC OF MOSQUITOES

Mosquitoes are classified into two subfamilies Anophelinae and Culicinae, which are divided into a number of tribes (Table 1). Currently, 112 genera of mosquitoes are known around the world. The subfamily Anophelinae includes three genera, while the subfamily Culicinae includes 109, divided into 11 tribes (<http://mosquito-taxonomic-inventory.info/>). In Europe, seven genera (including 96 mosquito species) were reported until 2000 (Snow and Ramsdale 1999) (Table 1).

Recently, changes were published within the tribe *Aedini* (Reinert 2000, 2001, Reinert et al. 2004) that led to a scientific debate and confusion since many names are used for a single taxon. Here we use the traditional names, i.e. *Aedes* is considered as the genus, and *Ochlerotatus* and *Rusticoidus* as subgenera for the *Ochlerotatus* spp. sensu Reinert (Reinert 2000), the same with *Aedes (Finlaya) geniculatus* for *Ochlerotatus (Finlaya) geniculatus* sensu Reinert (Reinert 2000), and with *Aedes (Stegomyia) albopictus* sensu *Stegomyia albopicta* sensu Reinert (Reinert et al. 2004).

Table 1. Classification of the mosquito genera reported in Europe

Phylum	Arthropoda						
Class	Insecta						
Order	Diptera						
Suborder	Nematocera						
Infraorder	Culicomorpha						
Superfamily	Culicoidea						
Family	Culicidae (Meigen, 1818)						
Subfamilies	Anophelinae	Culicinae					
Tribes	Anophelini	Aedini	Culicini	Culisetini	Mansoniini	Orthopodomyiini	Uranotaeniini
Genera	<i>Anopheles</i>	<i>Aedes</i>	<i>Culex</i>	<i>Culiseta</i>	<i>Coquillettidia</i>	<i>Orthopodomyia</i>	<i>Uranotaenia</i>

From (Snow and Ramsdale 1999)

2.2 LIFE CYCLE AND MORPHOLOGY OF MOSQUITO

We are used to think of mosquito as a flying insect, but actually its life is related to air only for the adult form, whereas the juvenile forms live in water. The mosquito goes through a complete metamorphosis, like the other Diptera, colonizing two totally different habitats: air and water (Figure 1). The aerial form of the adult is followed by the subsequent aquatic forms of eggs, four moulting larval instars, and pupa.

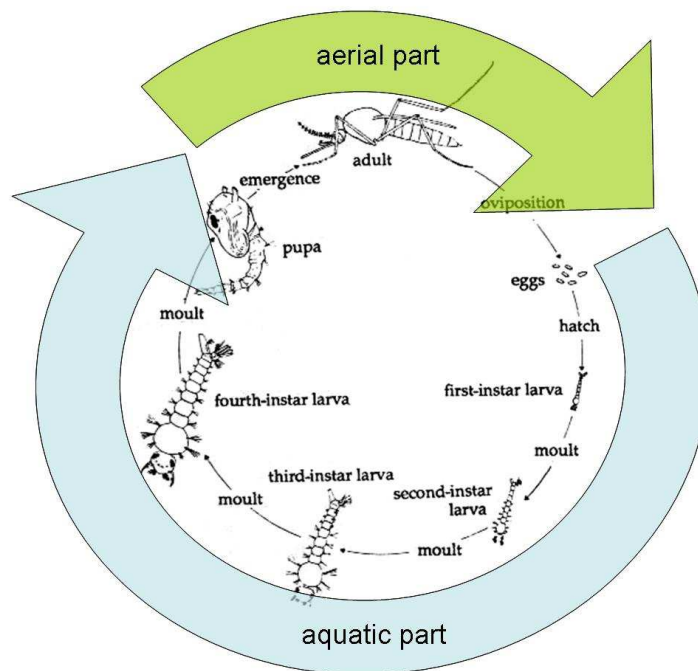


Figure 1. Schematic life cycle of mosquito (after Snow 1990)

2.2.1 EGGS

Usually 2-4 days after its insemination a female lays between 50 and 500 eggs (Figure 2) on the surface of still water or on solid surfaces close to the water level. The eggs have a tapering form, are dark and less than 1 mm long. The embryo is surrounded by a protective shell named chorion which shows different surfaces depending on the species. In *Aedes* species the chorion is resistant to dehydration and most species of this genus have an overwintering system that allows eggs to overcome extreme temperature conditions. In fact *Aedes* females lay their eggs on solid surfaces close to the water's edge. Those eggs can

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resist in the soil for 2-3 years and hatch once they are covered by water. This hatching is not simultaneous, only a part of eggs hatch with the first overflow, the other part will wait for subsequent floods, that means that eggs of the genus *Aedes* can support periods of dryness, while the chorion of eggs belonging to other genera can easily be damaged by desiccation, therefore these eggs have to be laid onto the water surface and hatch as soon as possible. *Anopheles* females lay single eggs, which are provided by expansions of the exochorion filled up with air called floats (Figure 2). Females belonging to the genera *Coquillettidia*, *Culex* and *Culiseta* instead lay their eggs in batches forming rafts of several hundreds of eggs locked together in a boat-shaped structure. In this case, each egg has a hydrophilic part, which lay on water, and a hydrophobic one. At the time of hatching the larva will break the shell and escape from a cap opening on the top of the egg (Marshall 1938).

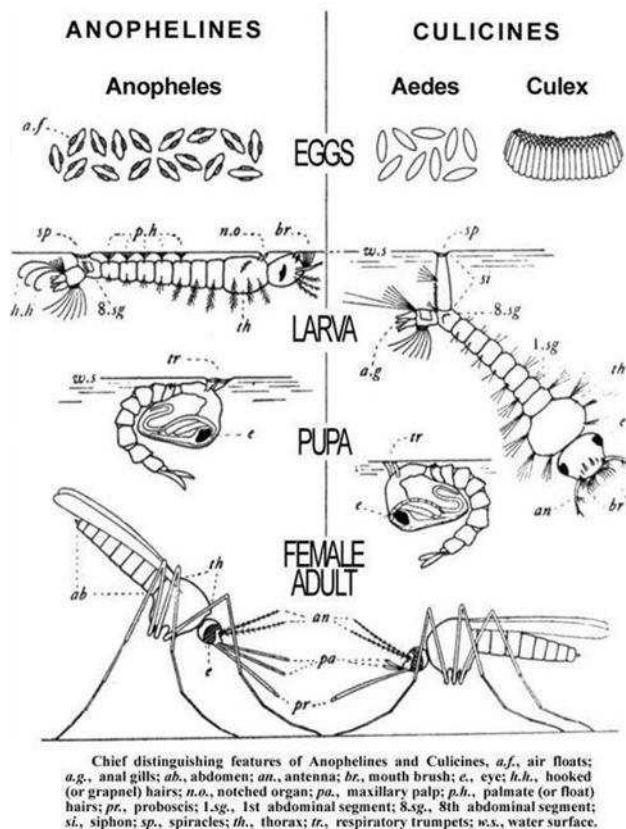


Figure 2. Main differences in juvenile and adult forms between the Subfamilies Anophelinae and Culicinae (Marshall 1938)

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2.2.2 LARVAE

Larvae (Figures 2 and 3) are growing through four moults. Their size goes from few millimetres at the first instar to about 1 cm long at the fourth. They have a soft body except for the part of the head and the respiratory siphon, which are covered by a sclerotized cuticle. Therefore these parts can change size only by moulting whereas the rest of the body grows continuously from one instar to the other. The head of larvae presents short antennae, primordial compound eyes of the future adult and a smaller simple larval eye located behind them. With “mouth brushes” they create water currents for feeding on food like microorganisms, algae, protozoa and detritus. Larvae are filter feeders by filtering particles in the water column. In addition larvae of the genus *Aedes* also browse their food by scrapping submerged substrates. Some larvae, for example of the genera *Aedes* and *Culex*, are also predators of other insects, mostly other mosquito larvae.

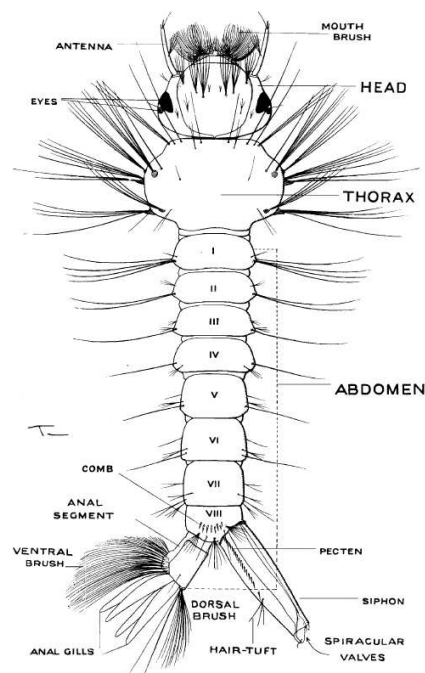


Figure 3. General outline of mosquito larvae (Marshall 1938)

The movement of larvae is provided by the mouthparts that create propulsion, setae, present on the thorax and abdomen, and by body contractions that create their typical

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jerky and wiggling movements. Larvae breathe air through a siphon present in Culicinae at the end of the abdomen or with spiracles present on the dorsal surface in Anophelinae. The position of the respiratory structures conditions the position under the water surface of the two subfamilies (Figure 2): Anophelinae are suspended horizontally, whereas Culicinae hang head downwards. In the genus *Coquillettidia* the siphon is modified for piercing submerged part of aquatic plants, through which they can obtain the oxygen. In this way, larvae can live submerged attached on aquatic plants, which allows them to escape predators like fishes. The duration of larval development depends on temperature and is related to the species. Usually, larval development in species that do not overwinter will last 2-3 weeks in temperate climate during springtime, while in summertime only one week is necessary to get transformed into pupae.

2.2.3 PUPAE

Pupae is the last aquatic phase - which usually lasts about two days - before metamorphosis into the adult form. Some larval organs like the alimentary canal are destroyed during the metamorphosis while others like heart and fat body are maintained. The pupa has a comma form (Figure 2) due to the cementation of the head with the thorax in a cephalothorax followed by an abdomen ending with two large paddles. The abdomen has retained the larval musculature, that with the paddles provides an efficient forward jumping propulsion. In the mesothoracic part, two spiracles with the form of trumpets are present for breathing atmospheric oxygen. Pupae do not feed and they cannot overwinter. When the adult is fully formed the cuticle on the thorax splits and the adult slowly emerges. Usually males emerge a day before females and form swarms where the female enter and get inseminated. A female needs to be inseminated only once in her life, because she can store sperm in spermathecae sufficient to fertilize several egg batches.

2.2.4 ADULT

The morphology of an adult mosquito is shown in Figure 4. The length of adult varies, but is rarely larger than 1.5 cm and weight up to 2.5 mg. All mosquitoes have slender bodies with three clearly distinguishable segments: head, thorax and abdomen. The mosquitoes differ from other Nematocera by having a proboscis always longer than the thorax. The head is specialised for receiving sensory information and for feeding. The surface of the head is almost completely occupied by two compound eyes. In addition, there are ocelli, which are simple photosensitive eyes to detect variations of light. The antennae are composed of 15 segments. The ones at the base of the antenna are responsible for the auditory function whereas the setae on the other part of the antenna have chemical and mechanical receptors. Antennae of females are important for detecting host odours. They can detect carbon dioxide released from their preys from about 100 m of distance, and breeding sites, while males have noticeably bushier and contain auditory receptors to detect the characteristic buzzing of the females. Mosquitoes feed on dissolved sugary fluids like nectar and plant juices to obtain the energy they need, while only females risk their life during blood meals to obtain protein for egg production.

The visible mouthpart of the mosquito is the proboscis, composed by a labium externally, which encloses the rest of the mouthparts: two mandibles, two maxillae, the hypopharynx and the labrum. On the top of the labium is the labellum, which is presumably responsible for the search of easily accessible blood vessels (Clements 1992). The mandibles and the maxillae are used for piercing the skin, while the saliva with anticoagulant and capillary dilators, to prevent clotting and to fast attract blood, is introduced through the hypopharynx. Blood is sucked by the labrum. Beside the proboscis there are the maxillary palps, food sensorial receptors, which are as long as the proboscis in the male and in the female of Anophelinae, while in Culicinae maxillary palps are much shorter. Not all the mosquitoes need blood for their egg production, for example the genus *Toxorhynchites*, present in Australia, contains enough protein from its larval stage. These mosquitoes have

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a so-called autogenous reproduction, whereas the other reproduction with the blood meal is called anautogenous. There are as well some species of the genus *Culex*, which are able to have a partially autogenous reproduction, without need of blood for their first cycle of egg production, permitting this way a faster increase of their progeny.

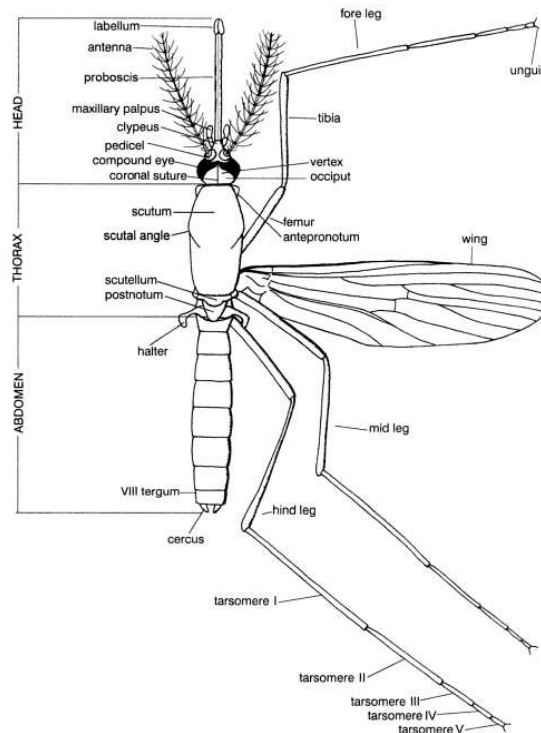


Figure 4. General outline of female Culicinae mosquito (after Marshall 1938) (Becker et al. 2010)

The thorax is specialized for locomotion. Three pairs of legs, a pair of wings and a pair of halteres, small wing-like organs used for steering, are attached to the thorax. The legs are composed of femur, tibia and 5 tarsomeres ending with nails. Wings have a lengthened oval form traversed by characteristic longitudinal and transversal veins. The abdomen is specialized for food digestion and egg development, in fact it can contain three times of the mosquito weight in blood, which is digested overtime (Clements 1992). The abdomen is divided into eleven segments. The last two ones are modified for the functions of mating and discharge of faeces, while by the females they serve to lay eggs as well.

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From their standing position it is fairly easy to distinguish the two subfamilies (Figure 2) of mosquitoes: in Anophelinae proboscis and body stay in one axis showing the mosquito completely stretched out horizontally with the substrate, whereas in Culicinae proboscis and body are in two axes with the abdomen more or less parallel with the substrate.

Life duration of females is depending on humidity and temperature conditions, whose optimum is related to the species. Globally, female life can last about one month (Delatte et al. 2009, Vrzal et al. 2010, Puggioli et al. 2013) during that time a female can lay several times eggs.

The biting time of mosquitoes varies according to the genera: *Culex* feed from dusk until a few hours after dark, *Anopheles* at dusk and dawn whereas *Aedes* day and night. The host preference is related to the species, but in general *Culex* prefers birds to humans, *Anopheles* tends to feed on humans and cattle, rather than other warm-blooded animals. Most *Aedes* are considered very aggressive to humans (Schaffner, Geoffroy, et al. 2001). Adults stay below vegetation to avoid dehydration when they are not active.

2.3 MOSQUITO HABITATS

Mosquitoes can colonize all sorts of still waters. The water has to be still, because larvae do not have tools to withstand running water, like for example caudal hooks present in other haematophagous Diptera like Simuliidae (Nematocera). Moreover, the emerging of adults is a very delicate phase, where the adults balance in equilibrium on the exuviae of the pupa, and water movements can make them fall and drown.

Culex and *Anopheles* species are permanent water mosquitoes, which lay their eggs on the surface of standing water at the edges of lakes and ponds and among the vegetation in swamps and marshes. Species of the genus *Aedes* breed instead in temporary waters: their eggs can survive to drying in soil or attached to artificial supports and hatch once flooded by water. Depending on the mosquito species, the water can have different degrees of salinity, for example *Ae. detritus* and *Ae. caspius* can live in brackish waters, or more or less organic components, i.e. *Cx. pipiens* and *Ae. albopictus* can live in fresh water or in polluted drains. The optimum water temperature for larval development is related as well to the

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species, snow-melt mosquitoes like *Ae. cantans* or *Ae. cataphylla* start their development once in pools formed from snow melting of heavy rain, whereas most of the other species develop between 10°C to 30°C and their aquatic period will last from 3 weeks to 1 week with increasing temperature conditions. The water can be permanent or temporary, for example floodwater mosquitoes like *Ae. vexans* or *Ae. sticticus* develop from areas along rivers or lakes which are temporary flooded by water fluctuations. Their reproduction is massive causing extreme nuisance, because in such waters no predators like fishes are present. Many species breed in arboreal cavities filled with water, like the tree-holes mosquitoes *Ae. geniculatus* or *An. plumbeus*, but many of these species adapted to small amount of water available in urban settlements, like drums, used tires, pot dishes. This is the case of the exotic species *Ae. albopictus* and *Ae. japonicus* that are now mostly considered container-breeding mosquitoes (European Centre for Disease Prevention and Control 2012).

Different mosquito species can coexist in one habitat, this can lead in some cases to predation among larvae. Usually natural environments offer a broader spectrum of species, and in those habitats predators are numerous: fishes or other invertebrate larvae in water and birds, bats, spiders and other animals in the air. In urban settlements the number of predator species is more limited. For example, in Ticino *Cx. pipiens molestus* can more or less grow undisturbed by predators. In addition, in natural environments mosquitoes may locally develop without larval predation. This is the case, for example, at the natural reserve of the Bolle di Magadino in Canton Ticino where the floodwater mosquitoes, e.g. *Ae. vexans*, can create nuisance to the surrounding urban settlements (Fouque et al. 1998) and breed in the absence of predators in temporary ponds created by the change of the level of the lake Maggiore.

2.4 MOSQUITO-BORNE DISEASES (MBD)

2.4.1 TRANSMISSION

Mosquitoes can transmit several microorganisms like bacteria, nematodes, protozoans and viruses. The transmission can be mechanical or biological (Chamberlain and Sudia 1961).

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Fenner and Day (Fenner and Day 1952) showed that mosquitoes were involved in pure mechanical transmission of fowlpox, disease of poultry, and myxomatosis, disease of rabbits, in the same manner the species *Ae. vexans* is involved in the porcine reproductive and respiratory syndrome virus (PRRSV) transmission (Otake et al. 2002). The biological transmission is more complex and is strictly related to the blood-sucking activities of the mosquitoes. This way they acquire from a vertebrate host the pathogen or parasite, which has to replicate and/or develop in the mosquito itself and can afterwards be injected in another host during the next blood meal. This is the case of the agent of malaria, *Plasmodium* sp. or nematodes causing filariasis or arthropod-borne-viruses (arboviruses) like dengue. In case of high level of viraemia there can be both mechanical and biological transmissions of the same virus, but mechanical transmission is a mere occasional occurrence, which has no particular epidemiological significance (Chamberlain and Sudia 1961). The diseases caused by pathogens that co-evolved with the mosquitoes are named mosquito-borne-diseases. The mosquito plays a role in the reproductive cycle of the pathogens and act as a vector of the disease. The transmission can be horizontal, i.e. the vector transmits the pathogen from one vertebrate to another, or can be vertical, i.e. some arboviruses can pass from one generation of mosquito to the next one by transovarial transmission. This mechanism contributes to maintain the virus even when adult mosquitoes are not active. In fact this system has been detected in some species of mosquitoes that have overwintering eggs, i.e. of the genus *Aedes* (Watts and Eldridge 1975). If a mosquito species is capable to transmit mechanically or biologically, this species is considered vector for the pathogen. The vector competence is part of the vectorial capacity, which measures the efficiency of vector-borne disease transmission. Vectorial capacity considers as well possible frequency of interactions between host and vectors, such as density of vectors and hosts, bites per host, daily survival of vectors and extrinsic incubation period of the pathogen.

Not all species of mosquitoes bite humans, many mosquito-borne diseases concern only animals, e.g. Usutu virus which kills birds. Other mosquitoes take their blood meals both on animals and humans. In this case, they have a role of bridge-vectors, that is they can

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transfer the pathogen from a confined cycle among certain animals to another one, e.g. *Ae. vexans* biting birds, livestock and humans can transfer the West Nile virus from its bird enzootic cycle to secondary hosts like horses (epizootic) or humans (epidemic). Other well-known diseases like dengue and yellow fever (YF) have their sylvatic cycle among primates transmitted by some jungle mosquito species. These viruses were restricted to primates until humans, entering in the jungle, went into contact with the viruses transmitted by bridge mosquitoes biting both primates and humans. This permitted transfer of the viruses to urbanized areas where the vectors responsible for epidemic are *Ae. aegypti* and *Ae. albopictus*.

2.4.2 MBD IN THE WORLD

As previously mentioned, over 50% of the human world population is under the threat of mosquito-borne diseases, principally in tropical and subtropical region, crowning this way the mosquito as the most dangerous animal to human kind. This concerns mostly malaria with around 200 millions infections every year, with 584,000 estimated deaths in 2013, mostly among children under five years and old people (“WHO | World Malaria Report 2014” 2015). Infections with dengue virus are estimated to reach 50-100 millions annually in the world, including 500,000 cases of hemorrhagic fever, with about 22,000 deaths, mostly among children (WHO 2009). In over 100 countries endemic for dengue, again about half of the world population is concerned with this virus. Therefore dengue is nowadays the most important arbovirus, with a rapid increase in the last years (30-fold in 50 years) (WHO: Impact of Dengue, <http://www.who.int/csr/disease/dengue/impact/en/>).

2.4.3 MBD IN EUROPE

In Europe mosquito-borne diseases have been historically present for centuries. Malaria came with the decline of the Roman Empire and has been present until the beginning of the last century (Sallares et al. 2004, Lalchhandama 2014). Similarly in south Switzerland the Plane of Magadino remained the last Swiss area still affected by the disease (Galli-Valerio 1905). Subsequently, malaria disappeared from the region in the 1930's (Borrani

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1937). Currently, it may happen that some autochthonous cases occur in Europe around airports in relation to inadvertent transport of infected mosquitoes from endemic areas. Vectors belonging to genus *Anopheles* are still present in Europe, but the risk of malaria transmission is considered low. The majority of the 32'394 autochthonous cases of malaria reported on this continent concerned the Caucasus region of Eastern Europe (Gratz 2004a). Eritja et al. (Eritja et al. 2005) reported dengue and YF epidemics in Spain between the XVIIIth and the XIXth centuries. Those epidemics had *Ae. aegypti* as vector and the outbreaks were first related to coastal areas, where sailboats imported both the vector and the disease. Consecutively inland locations, such as Madrid could be affected as well. In Greece, between 1927-1928, a large outbreak of dengue occurred with a million cases and 1,000 deaths (Theiler et al. 1960, Adhami and Reiter 1998).

In continental Europe, other arboviruses of clinical importance circulate, namely Sindbis virus (SINV), Tahyna virus (TAHV), West Nile virus (WNV), Batai virus (BATV) (Hubálek and Halouzka 1999, Gratz 2004a). All those viruses cause influenza-like symptoms: e.g. febrile illness, headache, myalgia, arthralgia, etc. For SINV and WNV, vertebrate hosts are principally birds. For SINV there are no case of human mortality reported, whereas WNV mortality in humans can reach 5-10%, mostly in persons >60 year old, and 25% in horses; both are considered secondary hosts for the virus. TAHV has as vertebrate host principally lagomorphs and cause no mortality in humans, the same for BATV but here the principal hosts are domestic pigs, horses and ruminants.

Dirofilariasis are nematodes transmitted by mosquitoes. In Europe the main vectors are *Ae. caspius* and *Ae. vexans* (Gratz 2004a). These nematodes are accidental human pathogens. *Dirofilaria immitis* (canine heartworm), whose principal hosts are dogs, migrates from an infected mosquito into pulmonary arteries of the host reaching the heart causing embolism, whereas principal hosts for *D. repens* are dogs and cats (Pampiglione and Rivasi 2000). In this case the nematode causes subcutaneous infections. Clinical effects on humans are cutaneous or intraorbital nodules (Gratz 2004a).

2.5 INVASIVE MOSQUITOES: *Aedes albopictus*

Invasive mosquitoes are species that have been introduced into an ecosystem and which have been able to get established causing an environmental, economic or human health impact (European Centre for Disease Prevention and Control 2012). In the last decades, the best-known exotic invasive mosquito is the tiger mosquito *Ae. albopictus*. This species native of Southeast Asia was originally relegated to the forested areas, where it breeds in tree holes, broken cane or coconuts. When urbanisation entered into contact with natural environments, new breeding sites were offered to this species like drums, buckets, small pots, used tyres, catch basins, etc. and blood meals were easily available from humans and domestic animals. This species takes its blood meals at daytime on almost any vertebrate. In fact *Ae. albopictus* bites humans, mammals, birds, batrachians or reptiles.

From Asia in the last Century the so-called tiger mosquito managed to spread worldwide throughout Africa, United States of America and Oceania (Hawley 1988, Gratz 2004b) mostly passively transported as immature stages in artificial containers, especially used tires (Moore et al. 1988, Knudsen 1995, Moore and Mitchell 1997).

The global transportation of goods permitted its arrival as well to the Old Continent. In Europe, the tiger mosquito was reported for the first time in Albania in 1979 (Adhami and Reiter 1998) where probably the commerce with China permitted its entrance. However Italy was the first country where its rapid spread was documented (Dalla Pozza, et al. 1994, Knudsen et al. 1996). The initial detection was reported in the city of Genoa in 1990 (Sabatini et al. 1990) and the first evidence of its establishment was observed in the Veneto Region in 1991 (Dalla Pozza and Majori 1992). In this region the spread started from a used tires deposit dealing with the United States, where a population of this mosquito was discovered in Texas in 1985 for the first time (Sprenger and Wuithiranyagool 1986, Dalla Pozza, et al. 1994) and few years after in several discarded tire deposits dealing with North Asia all over the country (Hawley 1988). Similarly the dispersal of *Ae. albopictus* immatures in Italy was mainly favoured by the trade of used tires (Romi and Majori 1998). In addition road traffic offered passive transport over long distances for adults (Di Luca et al. 2001). In

ten years, dispersal and climatic conditions facilitated the rapid spread to most regions in North and Central Italy (Romi et al. 1999). At that time, a few surveys started with consequent control measures on *Ae. albopictus*, i.e. around deposit for used tires in the Regions of Emilia-Romagna and Piedmont (Bellini 1999), as well as in the touristic municipality of Desenzano del Garda, in the Province of Brescia (Bellini et al. 1997). Elsewhere in Europe the tiger mosquito was detected locally in Northern France in used tire stock, probably coming from USA or Japan (Schaffner and Karch 2000). Control measures were undertaken and no further spread was documented.

2.6 MOSQUITO FAUNA OF SWITZERLAND

In Switzerland concerns on mosquitoes occurred until the beginning of last century when malaria was eradicated (Borrani 1937, Briegel et al. 2002), otherwise Switzerland is not considered a land of mosquitoes: there are no large flooded areas like the ones along the Rhine river in Germany or along the Danube in Central and Eastern Europe. In addition, most of the marshy areas have been drained at the beginning of last century and in mountainous environment mosquitoes may be present but their density is so low that they do not cause important nuisance for residents. The rice growing activities can cause extreme mosquito nuisance, as it is the case e.g. in Italy, but these agricultural activities are not very extended in our country. Hence mosquitoes are mostly found in pounds or in temporary water areas present in woods or wetlands, mostly belonging to protected areas as well as in urban environment (catch basins, all sort of recipient, etc.).

The first mosquito surveys at a national level focused on the genus *Anopheles* because of its role in malaria transmission (Galli-Valerio 1901, 1917). Other studies were initiated at the end of last century to control nuisance due to proliferation of human-biting mosquitoes like *Ae. vexans* in wetlands in Valais (Raboud 1980), around the lake of Gruyère (Lüthy 2013) and in Ticino in the natural reserves of the Bolle di Magadino and Maggia River (Focarile 1987, Lüthy 1987, Fouque et al. 1998).

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The general Swiss mosquito fauna at the beginning of the 1970's is reported in Briegel (Briegel 1973). The mosquito fauna of Switzerland was updated again in the Swiss mosquito checklist published by Briegel (Briegel 1998).

Table 2. Mosquito species reported in Switzerland and microorganisms reported in these species in Europe until 2000.

Species of the Swiss checklist (Briegel 1998)	Mosquito-borne pathogens (reported in EU) with some impact on human health
<i>Anopheles (Anopheles) claviger</i> (Meigen, 1804)	BATV, TAHV
<i>An. (Ano.) maculipennis</i> (Meigen, 1818)	Plasmodium sp
<i>An. (Ano.) maculipennis</i> s.l. (Meigen, 1818)	BATV, Plasmodium sp, WNV
<i>An. (Ano.) messeae</i> (Falleroni, 1926)	Plasmodium sp
<i>An. (Ano.) plumbeus</i> (Stephens, 1828)	Plasmodium sp, WNV
<i>Aedes (Aedes) cinereus</i> (Meigen, 1818)	SINV, TAHV, WNV
<i>Ae. (Aedes) geminus</i>	SINV, TAHV
<i>Ae. (Aedimorphus) vexans vexans</i> (Meigen, 1830)	Nematodes, TAHV, WNV
<i>Ae. (Finlaya) geniculatus</i> (Olivier, 1791)	WNV, YFV
<i>Ae. (Ochlerotatus) annulipes</i> (Meigen, 1830)	TAHV
<i>Ae. (Och.) cantans</i> (Meigen, 1818)	TAHV, WNV
<i>Ae. (Och.) cataphylla</i> (Dyar, 1916)	
<i>Ae. (Och.) communis</i> (De Geer, 1776)	BATV, TAHV, SINV
<i>Ae. (Och.) intrudens</i> (Dyar, 1919)	
<i>Ae. (Och.) pullatus</i> (Coquillett, 1904)	
<i>Ae. (Och.) punctator</i> (Kirby, 1837)	BATV, TAHV, WNV
<i>Ae. (Och.) riparius</i> (Dyar, 1919)	
<i>Ae. (Och.) sticticus</i> (Meigen, 1838)	TAHV
<i>Ae. (Russticoidus) refiki</i> (Medschid, 1928)	
<i>Ae. (Rus.) rusticus</i> (Rossi, 1790)	
<i>Coquillettidia (Coquillettidia) richiardii</i> (Ficalbi, 1889)	TAHV, WNV
<i>Culex (Culex) pipiens</i> (Linnaeus, 1758)	SINV, TAHV, WNV
<i>Cx. (Cux.) theileri</i> (Theobald, 1903)	SINV, WNV
<i>Cx. (Cux.) torrentium</i> (Martini, 1925)	SINV
<i>Cx. (Maillotia) hortensis hortensis</i> (Ficalbi, 1890)	
<i>Cx. (Neoculex) martinii</i> (Medschid, 1930)	
<i>Cx. (Ncx.) territans</i> (Walker, 1856)	
<i>Culiseta (Culicella) fumipennis</i> (Stephens, 1825)	
<i>Cs. (Cuc.) morsitans</i> (Theobald, 1901)	SINV
<i>Cs. (Culiseta) annulata</i> (Schrank, 1776)	TAHV, WNV
<i>Cs. (Cus.) glaphyoptera</i> (Schiner, 1872)	
<i>Orthopodomyia pulchripalpis</i> (Rondani, 1872)	

(Jetten and Takken 1994, Hubálek and Halouzka 1999, Lundström 1999, Gratz 2004a).

Abbreviations: Batai virus (BATV), Sindbis virus (SINV), Tahyna virus (TAHV), West Nile virus (WNV) and Yellow fever virus (YFV)

This includes thirty-three species known to be present before 2000. However, the presence of *Aedes geminus*, *Ae. riparus*, *Culex theileri*, *Culiseta glaphyoptera* and *Orthopodomyia pulchripalpis* needed to be confirmed according to Briegel (Briegel 1998). Mosquito species present in Switzerland are known vectors for various pathogens. That does not mean that they have the vectorial capacity for transmitting the diseases in Switzerland, because it depends on several conditions such as densities of mosquitoes and their possibility of interacting with their hosts. Table 2 shows the mosquito species listed in the Swiss checklist of Briegel (Briegel 1998) as well as mosquito-borne pathogens reported in these mosquito species in Europe before 2000.

2.7 MOSQUITOES IN CANTON TICINO

Until the beginning of this century, the knowledge on the mosquito fauna in the Canton Ticino, the Southern part of Switzerland, was limited. Only a few faunistic studies on mosquitoes were published.

The first investigations concerned in particular malaria vector mosquitoes belonging to the genus *Anopheles* (Galli-Valerio 1905, Borroni 1937). The Canton Ticino, in particular the flood plane of Magadino, remained the only malaria affected Swiss region until the 1930's. As reported by Galli-Valerio (Galli-Valerio 1905) and Borroni (Borroni 1937) the problem was eliminated by reducing larval habitats with environmental managements, such as drainages, embanking the main rivers and levelling the ground in order to reduce depressions where stagnant water could accumulate. Those interventions occurred as reaction to the main flooding of 1868. For Galli-Valerio (Galli-Valerio 1905) the disappearance of malaria can be attributed as well to the reduction of the costs of quinine and, consequently, of its more extensive use. Another factor was the changes in destination of emigrant workers of the Canton Ticino from Italian regions, where malaria was endemic, to other Swiss Cantons. On the other way, some resurgences of malaria in the Canton Ticino were related to Italian immigrant workers employed on construction of railways in the Canton. Besides Galli-Valerio (Galli-Valerio 1905) suggested the

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application of substances such as petroleum, ichthyol or lysoform on small water surfaces to kill immature stages of mosquitoes. At the same time a study, not focused on malaria vectors, enlarged the knowledge of the mosquito fauna in Ticino (Vogel 1931).

Table 3. List of mosquito species reported in Canton Ticino until 2000

Mosquito species reported in Ticino before 2000	Literature
<i>Anopheles (Anopheles) claviger</i> (Meigen, 1804)	(Borrani 1937; Briegel 1973; Fouque et al.1991; Galli-Valerio 1905; Vogel 1931)
<i>An. (Ano.) maculipennis</i> (Meigen, 1818)	(Borrani 1937; Briegel 1973; Focarile 1987; Fouque et al.1991; Galli-Valerio 1905)
<i>An. (Ano.) plumbeus</i> (Stephens, 1828)	(Borrani 1937; Briegel 1973; Focarile 1987; Vogel 1931)
<i>Aedes (Aedes) cinereus</i> (Meigen, 1818)	(Focarile 1987; Fouque et al.1991)
<i>Ae. (Aedimorphus) vexans vexans</i> (Meigen, 1830)	(Fouque et al.1991; Fouque et al.1998; Lüthy 1987)
<i>Ae. (Finlaya) geniculatus</i> (Olivier, 1791)	(Borrani 1937; Briegel 1973; Focarile 1987; Vogel 1931)
<i>Ae. (Ochlerotatus) annulipes</i> (Meigen, 1830)	(Fouque et al.1991)
<i>Ae. (Och.) cantans</i> (Meigen, 1818)	(Focarile 1987; Fouque et al.1991)
<i>Ae. (Och.) communis</i> (De Geer, 1776)	(Briegel 1973)
<i>Ae. (Och.) intrudens</i> (Dyar, 1919)	(Fouque et al.1991)
<i>Ae. (Och.) pullatus</i> (Coquillett, 1904)	(Borrani 1937)
<i>Ae. (Och.) punctor</i> (Kirby, 1837)	(Fouque et al.1991)
<i>Ae. (Och.) sticticus</i> (Meigen, 1838)	(Borrani 1937; Fouque et al.1991)
<i>Ae. (Rus.) rusticus</i> (Rossi, 1790)	(Focarile 1987)
<i>Cx. (Culex) pipiens</i> (Linnaeus, 1758)	(Borrani 1937; Briegel 1973; Fouque et al.1991; Lüthy 1987; Vogel 1931)
<i>Cx. (Cux.) theileri</i> (Theobald, 1903)	(Fouque et al.1991)
<i>Cx. (Cux.) torrentium</i> (Martini, 1925)	(Fouque et al.1991)
<i>Cx. (Maillotia) hortensis hortensis</i> (Ficalbi, 1890)	(Fouque et al.1991; Vogel 1931)
<i>Cx. (Ncx.) territans</i> (Walker, 1856)	(Focarile 1987; Fouque et al.1991)
<i>Cs. (Culiseta) annulata</i> (Schrank, 1776)	(Borrani 1937; Fouque et al.1991)
<i>Orthopodomyia pulchripalpis</i> (Rondani, 1872)	(Fouque et al.1991)

In the 1980's, mosquito studies on the natural reserve of the Bolle di Magadino were reinitiated when mosquito populations created nuisance for residents and an economic threat for tourism in the region of Locarno (Focarile 1987, Fouque et al. 1991). In parallel mosquito control programmes, based on the applications of selective mosquito bio-insecticide products based on *Bacillus thuringiensis israelensis (Bti)*, were established (Lüthy 1987, 2001, Fouque et al. 1998). In the remaining part of the region, no study was carried

out. Hence, globally, a total of twenty-two species were reported in Canton Ticino (Table 3), two of which (*Cx. theileri* and *Orthopodomyia pulchripalpis*) had to be confirmed according to Briegel (Briegel 1998) in the Swiss checklist of Culicidae.

2.8 THE GRUPPO DI LAVORO ZANZARE (GLZ)

Since 1988, in Canton Ticino, all the interest on mosquitoes was focused on the wetland area of the natural reserve of the Bolle di Magadino, where a specific mosquito working group, the Gruppo di Lavoro Zanzare (GLZ) was created. In 1988, cantonal authorities created GLZ to reduce floodwater mosquitoes that produced nuisance in the neighbouring villages and affected the tourism. Those floodwater mosquitoes started to be a nuisance problem on the Swiss side of lake Maggiore at the beginning of the 1980's. The barrage on the effluent of the Italian side created new water levels of the lake thereby producing new extensive breeding sites for the mosquito species *Ae. vexans*. Massive swarms of this species were considered responsible for the nuisance problem in the area of the Bolle di Magadino (Focarile 1987, Lüthy 1987, Fouque et al. 1991, 1998). The GLZ group coordinated control measures in the municipalities, where catch basins were treated regularly, and in the natural reserve, after springtime flooding caused by lake Maggiore's water level changes. VectoBac G® (Valent BioSciences Libertyville, IL, USA) an environmental friendly biocide based on *Bti* was used to control mosquito population and gave the expected results in reducing the nuisance (Lüthy and Patocchi 2014). This bacterium (*Bti*) during its sporulation produces specific proteins that kill mosquito larvae by their ingestion (Ben-Dov 2014), with no or reduced effects on no-target organisms (Boisvert and Boisvert 2000, Lacoursière and Boisvert 2004). Even if considered safe, the effects of an insecticide after long term treatments on a natural environment have to be investigated. The GLZ supported several studies on the faith and the effects of *Bti* treatments in the reserve. These studies showed that repeated treatments lead to accumulation of *Bti* spores in the soil of the reserve, where they were inactivated and remained (Chappuis 2002, De Respinis et al. 2006, Guidi et al. 2011). Moreover after more

than ten years of applications no sign of resistance to the biocide in mosquito populations was recorded (Flacio 2001).

2.9 OBJECTIVES OF THIS STUDY

At the beginning of 2000, knowledge on the mosquito fauna in Ticino needed to be re-evaluated and updated. In particular, it was important to screen all the natural wetlands with permanent and temporary water of the region, since they are natural habitats for mosquitoes. Further those areas have an important ecological relevance in biodiversity. In fact most of them are under cantonal or national protection, but being close to urban settlements, often residents complain about wetlands considered responsible for nuisance. Therefore, one aim of this work was to screen for the species of mosquitoes breeding in wetlands and the ones present in the surrounding villages of the canton Ticino, in order to identify the origin of nuisance. Assessing the source of the nuisance is essential to plan control measures.

Further knowing the species location and densities is also important to prevent risk of transmission of mosquito-borne pathogens. This need increased at the end of last century when West Nile virus (WNV) circulation along Europe re-emerged after the 1960's (Hubálek and Halouzka 1999). Scientists feared epidemic related to this arbovirus after the one that occurred in New York in 1999 (Nash et al. 2001). In Europe, this neuro-invasive virus was detected in horses in Italy in 1998 (Autorino et al. 2002) and in France in 2000 (McLean et al. 2002). WNV is an arbovirus that circulates between *Culex* species and birds, but that threatens human and horse health. In humans the virus remains mostly asymptomatic (60-80%). The symptoms are influenza-like and <1% of the infected persons may develop and encephalitic disease, with a fatality rate around 15%. In horses the mortality rate can reach 65% (Gratz 2004a). A vaccine is available for horses but not for humans. Long distance transport of this virus was attributed to infected migratory birds that transferred the virus to local birds through the bites of specific mosquito species. *Culex* spp. are considered responsible for the maintenance of the bird-to-bird enzootic

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cycle, but this virus can spill over to domestic animals such horses and human thanks to mosquitoes that can bite both birds and mammals, e.g. *Ae. vexans*.

The natural reserve of the Bolle di Magadino fits the risk of introducing WNV in Canton Ticino. In fact the Bolle di Magadino is a wetland of international relevance considering bird migration (belonging to the Ramsar convention) and the potential bridge vector for WNV, *Ae. vexans*, was considered the most representative mosquito of the wetland. Therefore it was of primary importance not only for the region of the Bolle di Magadino but also for the other wetland areas of the Canton to have an accurate knowledge of the mosquito fauna. Hence, our work also aimed to know where local mosquito species competent for arbovirus transmission were located in natural and/or urban environments in order to be able to collect them for further analysis for presence of arboviruses and then estimate a vectorial capacity in diseases transmission.

However, currently, not only local mosquito fauna is important. In fact, it is now well known that international travel and trade can import mosquitoes and mosquito-borne diseases by infected hosts. In addition, climate change can both affect the development and survival of the vector, as well as the development rates of the pathogens and therefore the transmission of diseases. This problematic is particularly relevant for *Ae. albopictus*, which is probably the best example of a “travelling” mosquito and a generalist vector of various arboviruses that threaten human health (Shroyer 1986, Hawley 1988, Guillet and Nathan 1999, Gratz 2004b) (Table 4).

Table 4. Known arboviruses infection in laboratory or in field collected *Ae. albopictus* and historical presence of arboviruses in Europe

Virus	Laboratory infection	Field positives	Presence in the Mediterranean
<i>Flavivirus</i>			
Dengue (all 4 serotypes)	x	x	x (past)
West Nile	x	x	x
Yellow Fever	x		x (past)
Japanese Encephalitis	x	x	
Israel Turkey Encephalitis			x
<i>Bunyavirus</i>			
Jamestown Canyon	x	x	
Keystone	x	x	
LaCrosse	x	x	
Oropouche	x		
Potosi	x	x	
Rift Valley fever	x		x
San Angelo	x		
Trivittatus	x		
Cache Valley	x	x	
Tensaw	x	x	
Tahyna			x
Batai			x
<i>Alphavirus</i>			
Westen Equine Encephalitis	x		
Eastern Equine Encephalitis	x	x	
Venezuelan Equine Encephalitis	x		
Chikungunya	x		x
Sindbis	x		
Mayaro	x		
Ross River	x		

Data known until 2005 (Eritja et al. 2005)

By the end of last century, Europe started experiencing invasion by new exotic mosquitoes like *Ae. albopictus*, which showed a rapid spread in urban settlements in neighbouring Italy, creating concern among scientists because of its vectorial competence for several arboviruses. The Canton Ticino is located on one of the main traffic axes connecting Italy to northern Europe, climatic conditions there are similar to the ones where the mosquito had spread in Italy. The risk of introduction with traffic and its establishment was realistic. Therefore the consultant of the GLZ, Peter Lüthy, pushed the group to start investigations on the presence of this species in the Canton in 2000 and a preventive surveillance system

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of *Ae.albopictus* was created. The surveillance system aimed to front the tiger mosquito at its arrival in the South of Switzerland from Italy.

The last aim of our work was to establish a surveillance system and to survey and control *Ae. albopictus* in Canton Ticino. This system had to focus on two major objectives: survey of the mosquito and control measures. In fact, surveying the mosquito was considered important to set up strategies and effective actions for its elimination or the slow down of its establishment. Keeping densities of this species under control was considered necessary to prevent autochthonous transmission of *Ae. albopictus*-borne diseases in case of introduction of the disease. Furthermore we aimed to reduce the nuisance for the residents of this very annoying day-biting mosquito by controlling the mosquito density and spread. We aimed to establish a mosquito control managed by the GLZ and appropriate authorities, which would minimise undesirable environmental contaminations caused by private interventions not sustained by knowledge of the mosquito ecology and correct use of insecticides. Finally, we intended to manage *Ae. albopictus* in such a way that it could have a favourable impact on the tourism, which is economically relevant for the Canton, similar to what happened with the successful management of the floodwaters mosquitoes in the area of the Bolle di Magadino (Lüthy 2001).

3 MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 SAMPLING LOCATIONS

Mosquitoes need water for reproduction, for example they lay eggs in pounds, temporary flooded areas (Figure 5.a.), tree holes, which can mostly be found in natural environments, or in all sorts of artificial recipients, like catch basins (Figure 5.b.), drums, which are characteristic of urban areas.

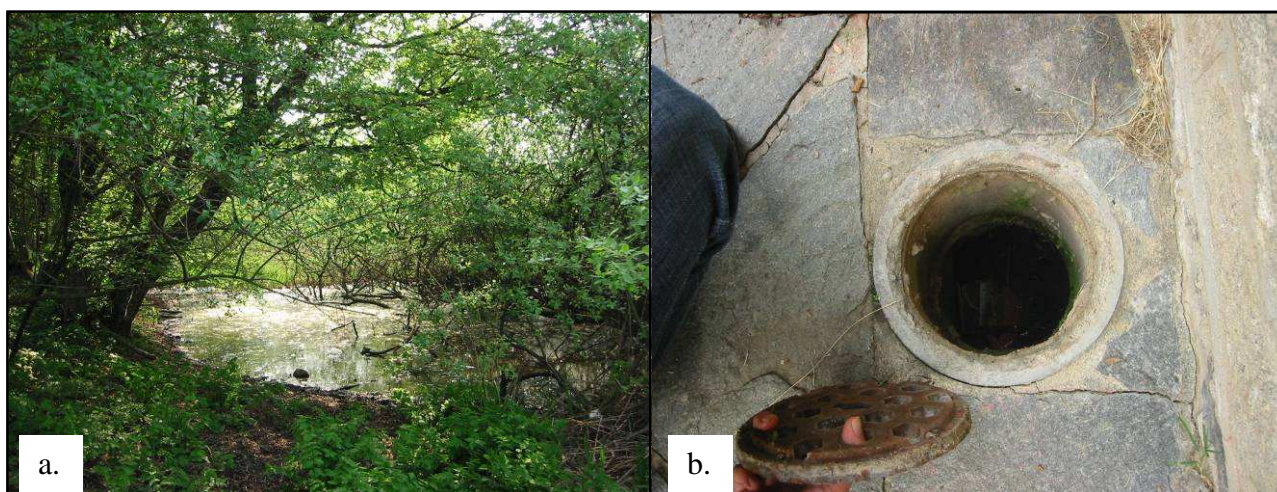


Figure 5. Examples of natural and artificial mosquito breeding sites: temporary water in wetland (a) and domestic catch basin (b.) (photos E. Flacio)

Knowing where eggs are laid and larvae and pupae develop gives indication on the origin of the breeding places, but adults move, some time far away, seeking their hosts for the blood meal. The dispersal can be active, depending on food and host availability, or the mosquitoes can be passively transported by wind or artificial tools, like cars, or in containers, that are displaced, e.g. used tires, which act as breeding places.

We checked aquatic environments in areas where mosquitoes could produce nuisance. Therefore the main natural environments were sampled (Figure 6), i.e. wetlands, known to be reproduction sites, as well as the surrounding urbanized areas to check if the mosquitoes creating nuisance to residents originated from the wetlands or the urban environments. To survey the tiger mosquito, *Ae. albopictus*, mainly urbanized areas were

controlled, but some natural environments, especially those close to villages infested by *Ae. albopictus* were sampled as well.

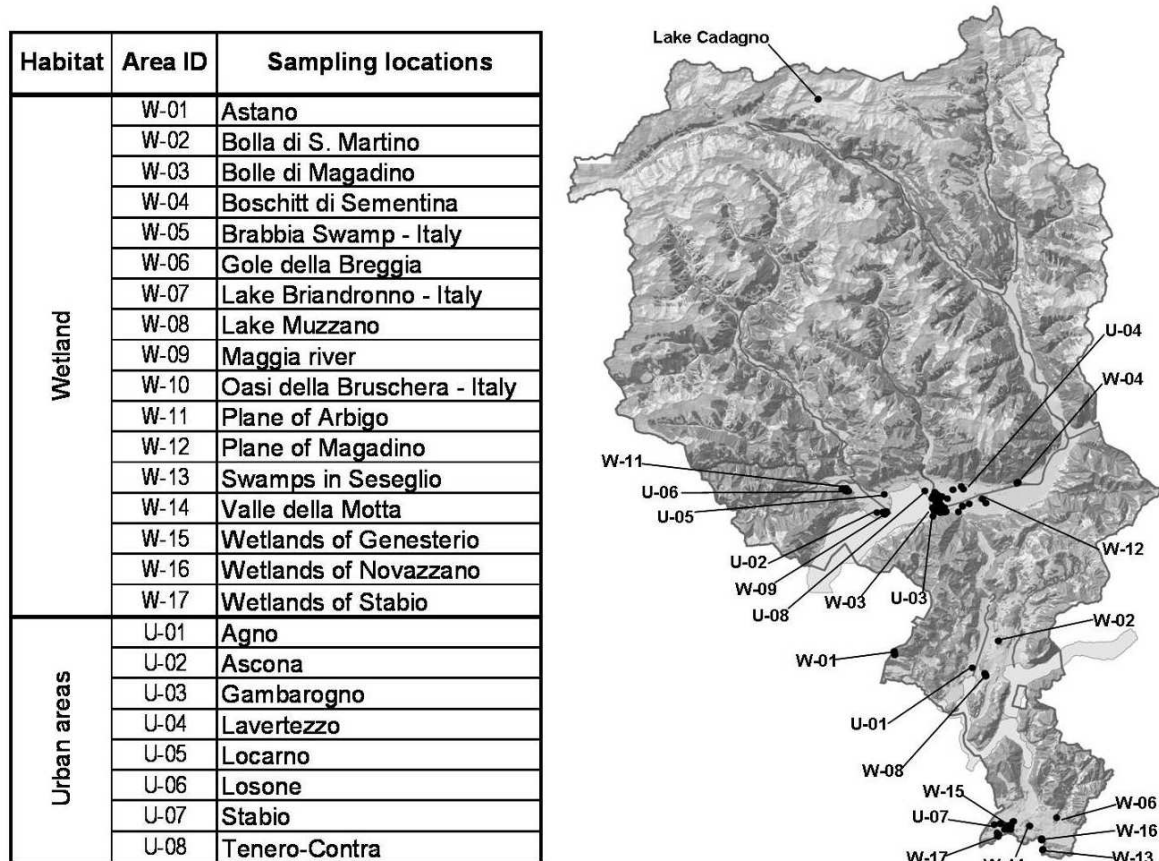


Figure 6. Sampling areas in natural and urban environments

3.1.1 NATURAL ENVIRONMENT

To have an overview on the mosquito fauna in Ticino, fourteen wetlands were controlled (Figure 6), among which eleven are protected (Ufficio federale dell’ambiente 2007; Ufficio della natura e del paesaggio 1993): Astano (2504¹), Bolla di S. Martino (2512), Bolle di Magadino (2314, 2299), Boschitt di Sementina (2302), Gole della Breggia, Lake Muzzano (2323), Maggia river (2333), Plane of Arbigo (2331, 3727), plane of Magadino (2304, 2310), swamps in Seseglio (2500), Valle della Motta, wetlands of Genesterio (2503), wetlands of Novazzano (2501) and wetlands of Stabio (2502, 2497) (Figure 6).

¹ In parentheses is shown the inventory number for wetlands of national or cantonal relevance

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Main relevance was given to the Bolle di Magadino and Maggia river because they are the main wetland in Ticino, historically “full of mosquitoes” (Galli-Valerio 1905, Borrani 1937), a Ramsar international wetland protection site, with an important relevance for bird migrations. From the 1980’s, measures in those areas are regularly undertaken to control swarming of mosquitoes causing nuisance to neighbouring villages (Lüthy 1987, 2001).

In order to compare the mosquito species of wetlands in Ticino and to prevent new introductions mosquitoes were sampled in three additional protected wetland areas located in Italy in the bordering province of Varese namely the Oasi della Bruschera (Protected area by regional laws n. 34934 of 19.07.1988), the Brabbia’s Ramsar swamp (Protection Area EUAP0323) and the lake Biandronno (Protection Area EUAP0308).

3.1.2 RESIDENTIAL ZONES CLOSE TO WETLANDS

To check if mosquito breeding in natural environments created nuisance to the surrounding villages, urban zones at a distance <500m were controlled. The residential area of Ascona close to the natural reserve Maggia river (U-02), Gambarogno (hamlet of Magadino) (U-03) close to the southern part of the Bolle di Magadino, camping places in the town of Tenero-Contra (U-08) next to the northern part of the Bolle di Magadino, the camping site of Losone (U-06) close to wooden pounds in the plane of Arbigo, the industrial areas of Stabio (U-07) close to the wetlands of Stabio (Figure 6). Some other urban zones away from wetlands, i.e. at a distance >500m, were checked for the species normally breeding there: the airport of Agno (U-01), Ascona (U-02), the residential area of the hamlet of Magadino (Gambarogno) (U-03), one house in Lavertezzo in the Plane of Magadino (U-03), Locarno (U-05), Tenero-Contra (U-06) and Stabio (U-07) (Figure 6).

3.1.3 *Aedes albopictus* IN URBAN ENVIRONMENT

In Italy, where *Ae. albopictus* started its European colonization, this mosquito was considered an urban breeding mosquito (Romi and Majori 1998), due to the enormous variety of small water containers that are available in those areas. Therefore we concentrate our survey in urban areas adopting the following criteria: the potential for the

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site to be an entry point for the mosquitoes by road traffic and the suitability of the site for the development of *Ae. albopictus*. We also took into consideration the priority of a location to be checked taking into account the nuisance and the impact of mosquito on sanitary and economic situations, i.e. schools, public parks, touristic places, etc. Thus hard shoulders on the highway, parking places, cemeteries, as well as private houses, etc were controlled. In addition the population was asked to notify suspected presence of tiger mosquito, which could be notified to the Gruppo di Lavoro Zanzare (GLZ).

3.1.4 *Aedes albopictus* IN NATURAL ENVIRONMENT

Although *Ae. albopictus* was considered an urban mosquito a survey was carried out in natural reserves (Figure 6): Astano (W-01), Bolla di S. Martino (W-02), Bolle di Magadino (W-03), Boschitt di Sementina (W-04), Lake Muzzano (W-08), Maggia river (W-09), Plane of Arbigo (W-11), plane of Magadino (W-12), swamps in Seseglio (W-13), wetlands of Genesterio (W-15), wetlands of Novazzano (W-16) and wetlands of Stabio (W-17). In addition a particular attention was given to some wooden locations close to villages colonized by this species to see if the mosquito could spread in those areas and breed.

3.2 MOSQUITO SAMPLING

To detect the presence of mosquito species all the developmental stages should be looked for: eggs, larvae, pupae and adults (Figure 7).

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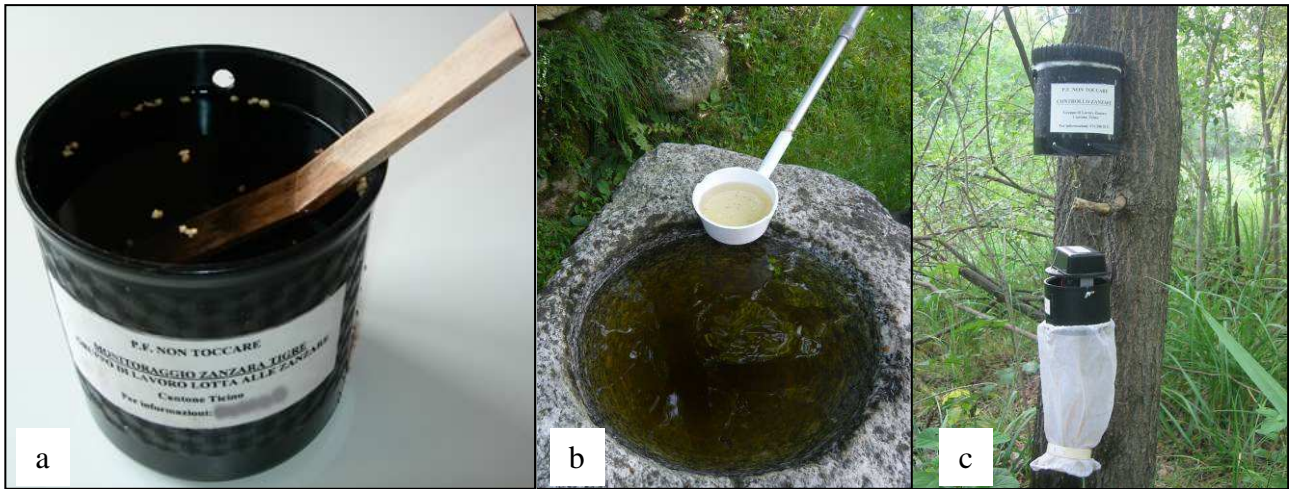


Figure 7. Examples of methods applied for mosquito sampling. a. Sampling eggs with an ovitrap built up for the survey on *Ae. albopictus* in Canton Ticino (photo A. Rose); b. sampling of larvae with dipper (photo E. Flacio); c. adult trap placed on the field (EVS CO₂-baited mosquito trap) (photo E. Flacio)

The developmental stage to be investigated depends on the straightforwardness and efficacy of the methodology adopted. In fact, for some species it is easier to detect the eggs, for others the larvae and by others the adult form. For example, by *Aedes* species that lay eggs in the ground like *Ae. vexans*, the eggs are difficult to sample. The soil close to water surface should be collected and then sieved (Silver 2008). In this case it is not evident to find the right place where eggs have been laid and then it is difficult after the sieving to have living eggs ready to hatch. Therefore it is easier to look after larvae or adults. Larvae of the genus *Coquillettidia*, stay under water attached to aquatic plants, therefore they are very difficult to detect. In this case it is worth to look after the adult form. Vice versa all forms of *Ae. albopictus*, a container breeding mosquito, can be sampled with different methodologies, but for an extensive survey system egg sampling with oviposition traps is the cheapest way, therefore this methodology has been privileged to others. Further every type of adult trap attract species in a different way (Lühken et al. 2014), therefore there is not a straight correlation in mosquito species densities with the use of a unique tool. Thus to have an overview on mosquito species in this work different sample methodologies were applied simultaneously.

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Still waters in urban and natural ecosystems were investigated to collect juvenile forms of mosquitoes, traps for mosquito adults were placed, and the presence of eggs of *Ae. albopictus* was checked.

3.2.1 EGGS

Ae. albopictus eggs were collected using oviposition traps (ovitraps) (Figure 7a). Ovitrap attract the female mosquito through the form of the container (black colour and the small size) and water. A support (the paddle) is added to allow the mosquito to lay its eggs, and eventually odours can be added. This support is then removed for analysis of the presence of the eggs, it is light coloured in order to facilitate the morphological detection of the eggs (black coloured) and should not have any repellent effect on the female mosquito. These ovitraps have been the main tool for the survey on *Ae. albopictus*. They are generally considered the easiest way to detect the presence of species belonging to the subgenus *Stegomyia*, i.e. *Ae. aegypti* (Linnaeus, 1862) and *Ae. albopictus*, (Romi 1996a, Silver 2008). Ovitrap detect eggs even at low mosquito densities, are not expensive and are easy to handle even by unskilled staff. To be used in a large scale survey a compromise between attractiveness for the mosquito (colour, shape, odours, persistence of water), safeness, i.e. not being breeding sites themselves, simplicity in handling and availability on the market has to be found.

We used black plastic containers of 1 l (Ramona Ø13/H12, Luwasa® Interhydro AG, Allmendingen, Switzerland) with an efflux hole on the top border (Figure 7a). A slat of steamed beechwood (200 x 26 x 6 mm) was placed as an oviposition support in an angular position in the plastic container, which was filled with tap water. Ten granules of VectoBac G® (Valent BioSciences, Libertyville, USA) were added in order to block the development of hatched larvae. The ovitraps were labelled with all the necessary information including phone number and the pledge not to remove it. Ovitrap were positioned close to or under vegetation or near buildings. An important factor was to select the most suitable place to position the ovitraps: it had to catch the mosquito at its arrival place, i.e. getting out from a vehicle, before finding several other breeding sites and/or it had to be placed where adults

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could easily live, i.e. presence of hosts for blood meal, shadow, etc. The traps were controlled bi-weekly. They were rinsed and filled with fresh water and VectoBac G[®] granules. The paddle was retrieved, labelled, wrapped into a plastic film, replaced and brought to the laboratory for examination of the presence of eggs.

3.2.2 JUVENILE STAGES

Juvenile stages can be sampled in water either directly with a Pasteur pipette with the tip cut off or using a standard pint dipper (model 1132, BioQuip Products, Rancho Dominguez, USA), consisting of a white (to contrast the larvae or pupae colour, which is brown or black) plastic container of 11 cm in diameter with a capacity of 350 ml (Figure 7b). It is important to sample without creating shadows on the water surface not to scare larvae and make them sink to the bottom. For every sampling site, water collection was repeated at least 3 times, each time in a different part of the pond in order to cover a total water surface of 30 m². In case of catch basins the sampling was also repeated three times but at intervals of 3-5 minutes from each tray in order to permit the larvae to reach the water surface again after sinking.

For sampling of *Ae. albopictus* larvae in deep containers, such as catch basins or drums, a fish net for aquariums can be used because larvae can remain at bottom for several minutes. Afterwards, the fish net has to be cleaned out in a recipient with water.

In case larvae had not reached the fourth stadium they have to be transported to the laboratory in cold conditions so they do not stress and die during the transport. The larvae are concentrated in a plastic container with a cap using a sifter or a pipette and the recipient was kept in thermic bag containing frozen freezer packs. Once in the laboratory larvae are kept at 28°C, and under 12 hours/ daylight in plastic trays filled up with tap water, feed with pulverized (using a mortar) fish food (TetraMin[®] Fish Food Flakes), in the presence of an aquarium aerator not to create mouldy water surface. Once larvae reached the fourth stadium, they were stored in 70% ethanol. Pupae were kept in the laboratory at room temperature until they emerge as adults under mosquito cages.

3.2.3 ADULTS

Traps for adult sampling have all the same principle: attracting the mosquito and sucking it out to a collector recipient. Light can be used as attractant, but it is not very specific. Mosquitoes, like other haematophagous insects, detect their prey following different chemical stimuli, like carbon dioxide, released with breathing, or body odours. Carbon dioxide can be added to traps for adults with a bottle or in form of dry ice whereas there are different products that simulate body odours.

For adult sampling, we used heavy duty EVS CO₂-baited mosquito traps (model 2801A, BioQuip Products) placed with the mosquito entrance at 1.5 m above ground level and dry ice (CO₂) was used as attractant (Figure 7c).

In some cases we used Mosquito Magnet® Liberty plus with either Octenol or Lurex™ or Lurex3™ as an attractant (www.mosquitomagnet.com). Traps were placed in the afternoon and removed the following day no later than midday. To catch *Ae. albopictus* adults, BG-sentinel traps (Biogents® AG, Regensburg, Germany) equipped with BG-Lure (Biogents® AG) and CO₂ as attractants were placed on the ground.

In this case traps were preferably placed in the morning and removed the following day at the same time. For instant catches an insect net or aspirator (Flashlight Aspirator, 2809C, www.bioquip.com) was used. To estimate nuisance Human Landing Collections were performed by estimating the number of females landing for 15 minutes (Silver 2008). Adults were killed by exposure to dry ice and stored at -20°C or dry for conservation purposes.

3.2.4 PERIOD OF SAMPLING

The period of sampling for the tiger mosquitoes was adapted to their known activity periods according to reports from Italy (Romi 1996a). The extensive sampling started generally at the beginning of May till the end of September, sometimes from April till the end of October and some more restricted samplings continued in the cold season from December till May in order to see if this species could be active during the cold season.

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The sampling period for the other species was chosen when mosquito and human activities matched i.e. in the warm season. Samplings were conducted between mid-April and end of October returning at least twice in the same sampling sites each season.

3.2.5 COMMUNITY PARTICIPATION

Because the trapping system for *Ae. albopictus* was insufficient to cover the whole territory, community participation was requested: residents were asked to report the occurrence of this species. The residents could report the presence of tiger mosquito in sending samples to the laboratory. Samples had to be sent in a small box to avoid disruption in shifting and origin, data of collection as well as telephone number had to be indicated. All notifications were followed by an answer of the expert of the GLZ to the residents.

3.3 MOSQUITO IDENTIFICATION

Mosquito adults and larvae were identified to the species level using morphological keys (Romi et al. 1997, Stojanovich and Scott 1997, Schaffner, Geoffroy, et al. 2001, Becker et al. 2003, Severini et al. 2009). When examining adults, only females were used.

Eggs collected with ovitraps were morphologically identified under a dissecting microscope according to Zamburlini & Frilli (Zamburlini and Frilli 2003). Morphological identification of eggs was regularly confirmed by larvae hatched from the eggs or by using the Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry technique (MALDI-TOF MS) (Schaffner et al. 2014). The preparation procedure for the MALDI-TOF MS analysis of eggs is shown in Annex 1.

3.4 DATA ANALYSIS

Data were organized in a relational database created with Microsoft Access®. For every sample following information was recorded: mosquito genus and species, mosquito developmental stage (egg, larva, female or male adult), number of individuals, date of the record and date of the analysis, sample methodology, person that did the sampling, name of the sampling position, its habitat description. Each record was geo-referenced (with

<https://map.geo.admin.ch>). All the data are available at the Centre Suisse de Cartographie de la Faune (CSCF) (www.cscf.ch). Further maps were created with ESRI ArcGIS®.

3.5 CONTROL MEASURES

Control measures for mosquitoes intend to limit or, in the best cases, eliminate the presence of the adult forms. The most effective control measures are the ones that act in a preventive way, that is to say against the juvenile stages (Figure 8 a-c). In fact, it is easier and more effective to operate against the aquatic forms, because normally water surfaces are limited and visible. Thus, if the surfaces are correctly managed the result is mostly achieved. In opposition, to strike the flying adult forms (Figure 8.d.) is more complicated, the results are temporary and there is a risk of impacting the environment unnecessary.

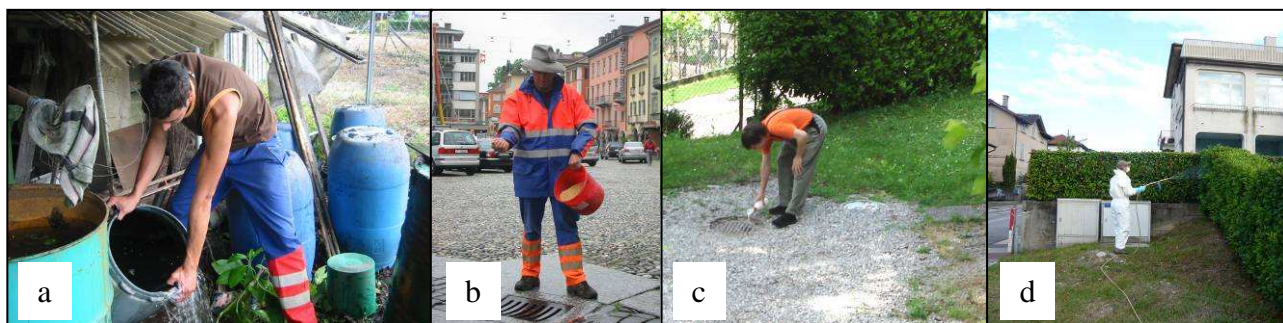


Figure 8. Control measures adopted against *Ae. albopictus*: a. removing breeding places; b. larvicide application in catch basins with *Bti*; c. larvicide application against *Ae. albopictus* in catch basins with diflubenzuron; d. adulticide application against *Ae. albopictus* on the vegetation around infested place (photos E. Flacio)

The control measures that act against the juvenile forms are: removing of the breeding sites, in case of container breeding mosquitoes such *Ae. albopictus*, and larvicide applications, whereas the insecticide application against adults is named adulticide.

To obtain the best results, a combination of these three measures should be applied (Figure 8.), but it is not always feasible, depending on the breeding site and the mosquito species to control. To reduce the presence of mosquitoes in natural environments only specific larvicides based on *Bti* are allowed and in case of a natural reserve a governmental permission is required. In urban environments, preventive actions, such as removal of

breeding sites and larvicide treatments are the ones recommended. Larvicide applications with *Bti* are permitted to everyone, whereas other larvicides can be applied only by professionals because they can affect other animals. There are no adulticides specific for mosquitoes, therefore those applications are recommended only in case of extreme nuisance and in particular aggregative places, such as schools, kindergartens etc, or in case of an epidemic risk. In any case, they have to be performed at dusk to prevent loss of bees, a protected species.

3.5.1 REMOVAL OF BREEDING SITES

Concerning *Ae. albopictus*, which is known to breed in small amounts of water, i.e. < 200 l (Hawley 1988), the containers that are not fixed, such as plant saucers, pot dishes, drums, buckets, tarpaulins, used tyres, bathtubs, etc., have to be removed or regularly emptied once a week during the warm season (Figure 8a).

Aiutateci a contenere la zanzara tigre!

Perché lotta alla zanzara tigre?

- Per salvaguardare la qualità di vita: è una zanzara molto aggressiva, punge ripetutamente, prevalentemente di giorno e colonizza l'ambiente urbano
- Per evitare il rischio di malattie: questa specie di zanzara è una potenziale portatrice di numerose gravi malattie

Cosa fanno i Comuni e il Gruppo cantonale di Lavoro Zanzare (GLZ)?

- Dal 2000 il GLZ svolge una sorveglianza preventiva sul territorio ticinese
- I comuni collaborano con il GLZ sia per la sorveglianza che per trattamenti contenitivi effettuati prevalentemente su suolo pubblico

Come riconoscerla

- Assomiglia ad una qualsiasi zanzara sia per forma che per dimensioni (ca. 0.5 - 1 cm), ma è nera con striature bianche (dimensioni reali, vedi immagine a lato)

Come si sviluppa

- Ogni zanzara femmina depone nella sua vita, che dura ca. 1 mese, centinaia di uova, le quali in una settimana diventano nuovi adulti, che a loro volta deporranno altrettante uova ciascuno, ecc.
- Le uova sono deposte prevalentemente in piccole raccolte d'acqua ferma: la quantità di un bicchiere è sufficiente!
- La zanzara tigre appiccica le sue uova al contenitore, la schiusa di queste non è simultanea, le uova possono sopravvivere a secco anche per parecchi mesi, superando anche l'inverno per poi schiudersi appena sono sommerse nuovamente con acqua

Come si diffonde

- Non è una buona volatrice (copre solo brevi distanze - meno di 100 metri), quindi si riproduce dove è osservata
- La zanzara tigre compie i suoi lunghi spostamenti entrando da clandestina a bordo di un qualsiasi mezzo di trasporto (autovetture, camion, container, ecc.)

Cosa potete fare voi

- La collaborazione dei cittadini è fondamentale!
- Per interrompere lo sviluppo bisogna toglierle l'acqua ferma: attorno alle nostre case ci sono numerosi contenitori che si possono riempire con l'acqua piovana o mediante irrigazione, diventando così potenziali focolai; nelle acque in movimento (corsi d'acqua, fontane, ecc.) le larve invece non riescono a sopravvivere
- Se osservate un qualsiasi caso sospetto contattate il GLZ!

Per ulteriori informazioni e/o segnalazioni:
<http://www.ti.ch/zanzare>

Gruppo cantonale di Lavoro Zanzare (GLZ) telefono: 091 935 00 46
Antenna Istituto di Microbiologia Applicata, via al Castello, 8957 Camébo, e-mail: dss-us.zanzaraligre@ti.ch

In che modo la si può combattere?

- Da aprile a novembre evitate di tenere all'aperto qualsiasi contenitore che possa riempirsi d'acqua: capovolgeteli e/o stocateli sotto una tettoia
- Sottovasi, piscine per bambini, abbeveratoi ecc. devono andare a secco almeno una volta a settimana
- Chiedete ermeticamente i bidoni per l'irrigazione quando non piove
- Nei biotopi e nei corsi d'acqua non si sviluppa la zanzara tigre: pesci e anfibii si nutrono delle loro larve
- La zanzara tigre si può sviluppare anche in piccole fessure nei muri ove ristagna acqua: colmatele di sabbia

❌

- Contenitori con acqua ferma vanno eliminati
- Sottovasi e piscine vanno vuotati settimanalmente
- Controllate che non vi siano ristagni nelle grondaie
- Colmate fessure nei muri con sabbia

✅

- Capovolgete o stocate al coperto i recipienti (vasi, secchi, copertoni, ecc.)
- Chiedete ermeticamente i bidoni
- Evitate di usare sottovasi
- Biotopi e corsi d'acqua naturali non producono zanzara tigre

❌

- Punti d'acqua ferma non eliminabili (tombini a griglia, pozzetti di grondaie, ecc.) possono essere trattati secondo le indicazioni del produttore (di regola settimanalmente) con un prodotto a base di *Bacillus thuringiensis israeliensis* (BTI)

Figure 9. Leaflet with general information on *Ae. albopictus* and control measures suggested to resident (www.ti.ch/zanzare)

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Both actions will avoid the development of the juvenile stages of the mosquito. This information was given in a specific leaflet (Figure 9), distributed door-to-door to residents by the municipalities and included in the web page of the GLZ and in most web pages of the municipalities involved in the surveillance system on *Ae. albopictus*.

3.5.2 LARVICIDE APPLICATIONS

Larvicide treatments, applied on water system producing mosquitoes, have the goal to control the juvenile stages to avoid emergence of adults. In this work, two main active ingredients were used: *Bti* and diflubenzuron.

3.5.2.1 *Bacillus thuringiensis israelensis*

Bti are bacteria that produce specific crystal proteins during their sporulation. These proteins, when ingested by a mosquito larva, interact with the gut epithelium causing its perforation and the consequent death of the mosquito. The advantage of applying *Bti* is that it is selective for mosquitoes with none or light reported effect on not-target organisms (Boisvert and Boisvert 2000, Lagadic et al. 2014), therefore it is considered the most environmental friendly larvicide.

This bio-insecticide is very effective, but having to be ingested, it does not target all the juvenile stages: first instar larvae and pupae are not affected. Another characteristic of insecticides containing *Bti* available on the Swiss market is that the effect occurs in 24 hours and that it does not last longer.

This insecticide is the only one permitted for applications in natural environments and the GLZ recommended its use to citizens for applications in private domains. In case of difficult weather conditions, like rain forecast, when there is a risk of rain-wash of larvicide from the catch basins to water systems, this insecticide is the only one used by the GLZ for extensive applications.

Bti was used for treatments in natural environments under the form of granules, VectoBac G® (Valent BioSciences). Helicopter and manual treatments occur on regular basis since 25 years in the natural reserve of the Bolle di Magadino (Lüthy and Patocchi 2014). In other

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wetlands the treatments were applied manually following the instruction on the product label.

VectoBac G[®] was also applied for treatments of catch basins (Figure 8.b.): 30 granules/catch basin (average water content of 50 l water). In the same breeding sites a liquid formulation of *Bti* (Solbac[®], Andermatt Biogarten AG) was applied with a concentration of 0.025 ml/catch basin. Solutions were applied either with a dosage bottle (Schnelldosierflasche cat. No. 3807, Semadeni Plastics Market AG, Ostermundigen, Switzerland) or with a knapsack sprayer.

Citizens were asked to apply tablets of *Bti* available in supermarkets (Solbac-Tabs[®], Andermatt Biogarten AG; Bio Garden Trauermücken-Stopp[®], Migros-Genossenschaftsbund; Coop Oecoplan Biocontrol Mücken-Tabletten[®], COOP) once a week from May till the end of September. The recommended treatment was 1 tablet per 20 catch basins, i.e. 0,5 tablet/ 0,5 l of water and 50 ml of this solution for each domestic breeding site. Indications for domestic treatments, e.g. list of supermarket selling the tablets, breeding sites to be treated and dilution of the product, were available on a leaflet and on the webpage of the GLZ (www.ti.ch/zanzare).

3.5.2.2 Diflubenzuron

Diflubenzuron is a synthetic product belonging to the category of insect growth regulators (IGR), which inhibits synthesis and deposition of chitin blocking this way the passage from an immature stage to the other and preventing the pupae to become adult. It is characterised by a low acute toxicity against mammals and a good safety margin with respect to non-target organisms including fish and birds (WHO 2001, 2005, 2006, KEM 2007). Another characteristic of diflubenzuron is its persistence of about one month (Dalla Pozza et al. 2004, Bellini et al. 2009).

This insecticide was used only for applications in catch basin (Figure 8c), where the biodiversity is restricted, and it had to be applied only in case of at least three days of sunny weather forecasts not to risk undesired contaminations of water systems. In case of

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rainy weather forecast diflubenzuron had to be replaced by *Bti*. The treatments were repeated monthly from mid-May till mid-September.

The only product containing diflubenzuron allowed on the Swiss market for treatments against mosquito is Device® SC-15 (Paradiffusion SA). Application consisted in 10 ml of a 2 ml/l solution either with a dosage bottle or a with knapsack sprayer in each catch basin (50 l).

3.5.3 ADULTICIDE APPLICATIONS

Adulticides were applied only against *Ae. albopictus* on the vegetation surrounding a positive area (Figure 8d) and only in particular cases, e.g. newly introduction, nuisance in aggregative places like schools or in case of epidemiological risk. All these treatments were executed joint with larvicide applications. Pyrethroids were used as insecticide.

Pyrethroids are a class of synthetic insecticides, with a chemical structure coming from the natural insecticide pyrethrums (contained in the flowers *Chrysanthemum cinerariaefolium* and *C. coccineum*). This class of insecticides has been built in to supply the natural one and to give a better stability to insecticides against UV destruction. In general pyrethroids are generic insecticide against insects, with as well repellent properties, low mammalian toxicity, a fast biodegradation and are broken by sunlight in one or two days (United States Environmental Protection Agency <http://www2.epa.gov/mosquitocontrol/permethrin-resmethrin-d-phenothrin-sumithrinr-synthetic-pyrethroids-mosquito-control>). They act as neurotoxins by contact to insects paralysing them to death. The pyrethroid used as adulticide is permethrin (one of the most biodegradable), rarely cypermethrin.

Since pyrethroid are not specific to a class of insects, they have been applied only with the intention to eliminate the tiger mosquito when this species was not yet established or in case of nuisance in aggregative and public places, like schools, retirement homes, public parks, etc. In addition in case of an epidemic risk, adulticide applications are foreseen.

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Adulticide applications were executed during the evening hours on the vegetation surrounding infested locations up to 2 m above the ground level with a backpack sprayer using permethrin (Matil[®], Maag AG) or cypermethrin (Cypermethrin[®], Sintagro AG), following dilutions recommended by the manufactures. Applications have to hit the target and not have side effects on non target organisms, therefore, in order not to affect the water, adulticide treatments were not performed close to rivers or lakes. In addition grasslands were barbed before so they did not need to be treated and edible vegetation was not concerned to avoid any health problem to the population. The adulticide and larvicide applications were performed on a radius of 100 m and 200 m around positive traps, respectively. All treatments were performed under the supervision and organisation of GLZ workers patented for use of biocide.

3.5.4 INFORMATION

In the control measures is included a targeted information campaign to the population to prevent the establishment of the tiger mosquito. Mass media like TV, radio and newspapers were used from the beginning of the survey to inform the population on the characteristics and risks related to *Ae. albopictus* during the summer season. Afterwards information on the situation about the mosquito and the measures undertaken by authorities were added, and citizens were invited to avoid having domestic breeding sites. This information belonged to an annual scheduled communication flow, which started after a press communication of the GLZ around May and was repeated in July and beginning of September in order to reach as many residents as possible. In addition, a GLZ web page (www.ti.ch/zanzare) was built containing downloadable general information on *Ae. albopictus*, annual reports of GLZ, FAQ format and leaflets targeting citizens. The leaflets were translated in several languages, i.e. Italian, French and German. They contained explanations on the risks related to the tiger mosquito (nuisance and vector capacity), the potential domestic breeding sites and the measures to undertake to prevent the reproduction of the mosquito (avoid, remove or treat breeding places). A list of *Bti* tablets available on the market and their suggested dilution were included. In all

documents phone numbers for notifications of the mosquito presence or request of information were available.

3.6 STATISTICS

Ae. albopictus surveillance eggs counts on ovitraps show excessive zeros especially during the first years when the mosquito is not widely spread. Therefore on analysis with egg densities, a zero inflated negative binomial function (ZINB) was applied, which considers these excess zeros as generated by a separate process from the count values and that the excess zeros can be modelled independently. The ZINB was then fitted with a generalised linear mixed model (GLMM) to relate egg's densities with several covariates, such as "year", "month" and "rain". In the GLMM, responses from a subject are thought to be the sum (linear) of so-called fixed and random effects. In our case the dependent variable was the total number of eggs on a single slat, while the explanatory variables were collection "year", "month" and "rain". "Year", "month" and "rain" were included as fixed effects, while a random effect term was added for the ovitraps, accounting for repeated measures in the same ovitrap. These permits to sort more accurate results with data that are not normally distributed.

4 RESULTS

4. RESULTS

4.1 PAPER 1

Flacio, E., L. Engeler, M. Tonolla, and P. Müller. In press. Spread and Establishment of *Aedes albopictus* in Southern Switzerland between 2003 and 2014: an Analysis of Oviposition Data and Weather Conditions. *Parasit. Vectors*.

4.2 PAPER 2

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4.3 PAPER 3

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4.4 PAPER 4

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4.1 PAPER 1

Spread and Establishment of *Aedes albopictus* in Southern Switzerland Between 2003 and 2014: an Analysis of Oviposition Data and Weather Conditions. *Parasites & Vectors* (in press)

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The rapid spread of the exotic mosquito *Aedes albopictus* that occurred in urban settlements in Italy by the end of last century (Romi et al. 1999) created concern among scientists because of its vectorial competence for several arboviruses (Hawley 1988). Used tires trade and traffic favoured the dispersal of this mosquito species over long distances (Romi and Majori 1998, Di Luca et al. 2001). The Canton Ticino has similar weather conditions to the ones where the mosquito had spread in Italy and is located on one of the major traffic axes connecting with Italy, therefore, in 2000 we decided to monitor the mosquito arrival and follow the distribution conditions in order to set up strategies and effective actions for its elimination or the slow down of its establishment.

This article is in press by Parasites & Vectors

1 **Spread and establishment of *Aedes albopictus* in southern Switzerland between**
2 **2003 and 2014: an analysis of oviposition data and weather conditions**

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22 **Abstract**

23 **Background**

24 The Asian tiger mosquito, *Aedes albopictus*, is a highly invasive mosquito species of public health
25 importance. In the wake of its arrival in neighbouring Italy the authorities of the canton of Ticino in
26 southern Switzerland initiated a surveillance programme in 2000 that is still on-going. Here we
27 explored the unique data set, compiled from 2003 to 2014, to analyse the local dynamic of
28 introduction and establishment of *Ae. albopictus*, its relative density in relation to precipitation and
29 temperature, and its potential distribution at the passage from southern to northern Europe.

30 **Methods**

31 The presence of *Ae. albopictus* was recorded by ovitraps placed across Ticino. In addition to
32 presence-absence, the relationship between relative egg densities and year, month, temperature and
33 precipitation was analysed by a generalised linear mixed model.

34 **Results**

35 Since its first detection in 2003 at Ticino's border with Italy *Ae. albopictus* has continuously spread
36 north across the lower valleys, mainly along the trans-European motorway, E35. Detailed local
37 analysis showed that industrial areas were colonised by the mosquito before residential areas and
38 that, afterwards, the mosquito was more present in residential than in industrial areas. *Ae. albopictus*
39 appeared sporadically and then became more present in the same places the following years,
40 suggesting gradual establishment of locally reproducing populations that manage to overwinter.
41 This trend continues as witnessed by both a growing area being infested and increasing egg counts
42 in the ovitraps. There was a clear South-North gradient with more traps being repeatedly positive in
43 the South and fewer eggs laid during periods of intensive precipitation. In the North, the mosquito
44 appeared repeatedly through the years, but never managed to establish, probably because of
45 unfavourable weather conditions and low road traffic.

46 **Conclusions**

47 Given the present results we assume that additional areas may still become infested. While the
48 current study provides good estimates of relative egg densities and shows the local and regional
49 dynamics of *Ae. albopictus* invasion, additional parameters ought to be measured to make an
50 objective risk assessment for epidemic disease transmission. The likelihood of *Ae. albopictus* to
51 further spread and increase in densities calls for continued surveillance.

52

53 **Background**

54 The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is considered the most invasive
55 mosquito species worldwide [1,2]. During the past 40 years *Ae. albopictus* spread from South-East
56 Asia to North and South America, parts of Africa, northern Australia, several Pacific and Oceanic
57 islands as well as many European countries [3,4]. Besides being an invasive species from an
58 environmental point of view, this mosquito also threatens human and animal health. *Ae. albopictus*
59 is a vector of chikungunya, dengue, zika virus as well as dirofilarial worms and, under laboratory
60 conditions, is able to transmit at least 27 arboviruses [3,5]. In continental Europe, several
61 autochthonous cases of chikungunya in Italy and France [6–8], and dengue in Croatia and France
62 [9–13], have been associated with *Ae. albopictus*.

63 It is assumed that the active flight range of *Ae. albopictus* is only a few hundred metres. For
64 example, Marini *et al.* [14] reported that the mosquito's average daily flight distance is only 119 m,
65 while, owing to its desiccation resistant eggs, *Ae. albopictus* is passively spread over long distances
66 through the international trade of used tyres and other artificial containers. At a more regional level,
67 adult mosquitoes are frequently stowed away in vehicles and subsequently displaced along roads
68 [3,15].

69 In Europe, *Ae. albopictus* was first recorded in Albania in 1979 [16] and later in Italy, in 1990 [17].
70 Less than a decade later it was established in the northern and central regions of Italy [18,19] from

71 where it spread further across Europe by means of public and private transport [20]. *Ae. albopictus*
72 is currently established in most Mediterranean coastal regions, including the islands, from Alicante
73 in Spain to Athens in Greece and across the whole of Italy [4,21]. In the North, the mosquito has
74 already been reported close to Paris and Strasbourg [22] and southern Germany along two
75 motorways connecting with southern Europe and in the cities of Heidelberg and Freiburg [23–25].
76 Fearing the possibility of *Ae. albopictus* introduction from northern Italy and the associated risk of
77 biting nuisance and disease transmission, the authorities of the Republic and Canton of Ticino, a
78 southern Swiss region bordering with Italy, initiated the surveillance of *Ae. albopictus* in the year
79 2000. In the beginning, surveillance focused on the trans-European motorway E35 that runs through
80 Ticino, connecting Italy with northern Europe. *Ae. albopictus* was first observed at a motorway
81 service area in 2003 [26]. Since then the surveillance programme, consisting of *Ae. albopictus*
82 monitoring and control, has been continuously expanded. The details of set-up and the history of the
83 Ticino surveillance programme are described in Flacio *et al.* [27].
84 Based on oviposition data collected between 2003 and 2014 we describe the local dynamic of
85 introduction, presence and establishment of *Ae. albopictus* and its relative density in relation to
86 precipitation and temperature in southern Switzerland.

87 **Methods**

88 **Study area**

89 Data were collected in the Canton of Ticino, Switzerland, between 3 July 2003 and 22 September
90 2014. The Canton of Ticino is located south of the Alps and is divided into eight districts five of
91 which were considered in this study: Mendrisio, Lugano, Locarno, Bellinzona and Riviera (Figure
92 1). The landscape is dominated by agriculture and forested hills, interspersed with lakes, rivers and
93 mountains that culminate at more than 3.000 m above sea level (a.s.l). Urban settlements are mainly
94 characterised by two-story houses, surrounded with private gardens and are mostly located in valley
95 floors below 400 m a.s.l. [28].

96 Over 62,000 commuters (data from 2014 [29]) cross the border between Italy and Ticino on a work
97 day. The canton is also an important passage for long distance road traffic with the trans-European
98 motorway E35 that runs from Rome (Italy) to Amsterdam (the Netherlands) through Ticino (Figure
99 1).

100 **Mosquito trapping**

101 All data were collected in the frame of a routine surveillance programme that has been initiated in
102 the year 2000 and then gradually expanded to cover larger areas in response to reported and
103 suspected presence of *Ae. albopictus*. The details of the programme and how it evolved over the
104 years are described in Flacio *et al.* [27].

105 Briefly, *Ae. albopictus* surveillance started by placing a few oviposition traps, hereafter called
106 “ovitrap”, at suspected entry points near the border with Italy such as motorway service areas and
107 locations with potential breeding sites like cemeteries with flower vases. Between 2005 and 2008,
108 the surveillance was gradually expanded, including industrial zones and larger car parks and public
109 areas in cities. In 2009 the initially targeted monitoring was extended to an area-wide surveillance
110 network covering the urban areas of entire municipalities [27].

111 Ovitrap consisted of 1.5 l black plastic containers filled with tap water into which a wooden slat is
112 placed as an oviposition substrate. To avoid larval development inside the trap, *Bacillus*
113 *thuringiensis* var. *israelensis* (*Bti*) granules were added to the water (for details see [27]). Ovitrap
114 were positioned on the ground under vegetation or near buildings, and slats, water and *Bti* granules
115 were replaced biweekly.

116 **Data recording**

117 The wooden slats were brought to the laboratory in Canobbio near Lugano and carefully inspected
118 under a stereo microscope with an 80x magnification. Eggs were morphologically identified
119 according to Zamburlini & Frilli [30] and counted. Morphological identification was regularly
120 controlled by hatching out eggs and by rearing the mosquitoes to late larval instars or adults to

121 confirm the species. Since 2013 batches of eggs have also been identified using Matrix Assisted
122 Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) approach [31].
123 Egg counts together with associated information, including trap position, date of collection and trap
124 condition were entered into an Access 2000 database (Microsoft Corporation Ltd., USA).

125 **Distribution and annual activity patterns**

126 Survey periods varied between years, ranging between calendar week 14 and 48 but always covered
127 the *Ae. albopictus* peak season (Additional file 1). *Ae. albopictus* females lay eggs that undergo
128 diapause during winter in more temperate regions [32]. To determine when overwintering eggs
129 hatch, slats with *Ae. albopictus* eggs that have been collected from positive ovitraps in calendar
130 week 40 in 2009 were left in the field. The slats were kept in mosquito breeders (similar to the ones
131 available on www.bioquip.com) made of 1,5 l transparent PET bottles. These breeders were then
132 placed inside the black ovitrap container. Mosquito breeders were checked for the presence of
133 larvae bi-weekly until hatching in 2010 [33].

134 In addition, to monitor mosquito activity during winter, ovitraps in locations that previously showed
135 high egg counts were left over winter and inspected each month for the presence of eggs. Eight
136 ovitraps were set in Lugano and eight traps in Chiasso from December to April 2012-2013 and
137 2013-2014.

138 **Analysis of egg counts**

139 For comparison between years, only data from the calendar weeks 22, 26, 30, 34 and 38 (May -
140 September) were considered (Additional file 1) to account for variations in the length of the
141 sampling periods between years. Depending on whether a trap was negative, sporadically or
142 continuously positive during three consecutive four-week periods, *Ae. albopictus* was deemed
143 “absent”, “introduced” or “established”, respectively. If an ovitrap was positive both in calendar
144 week 38 and in calendar week 22 of the following year, then the local population was assumed to

145 have overwintered in that location. To visualise these, data maps were drawn using ArcMap 10.2.2
146 (ESRI Inc., USA).

147 In addition to the presence-absence of *Ae. albopictus*, we also investigated the relationship between
148 actual egg counts and several potential covariates by fitting a generalised linear mixed model
149 (GLMM) with a negative binomial link function. In the GLMM the dependent variable was the total
150 number of eggs on a single slat, while the explanatory variables considered were the year and
151 month of collection, temperature and precipitation. These covariates were included as fixed effects,
152 while a random intercept was added for the ovitraps, accounting for repeated measures in the same
153 ovitrap. In the analysis, the weekly average temperature preceding one, two, three or four weeks as
154 well as the average temperature over one, two, three or four weeks prior inspecting the traps were
155 considered. For precipitation (i.e. rain) the cumulative volume per area was computed. Similar to
156 temperature, the weekly sum preceding one, two, three or four weeks as well as the cumulative
157 precipitation over one, two, three or four weeks prior inspecting the traps were considered. From the
158 full model including all above covariates only those that were significant ($\alpha = 0.5$) were retained in
159 the final model. Moreover, only one term for each temperature and precipitation was included in the
160 model. Meteorological data were retrieved from eight stations present in the study area (Additional
161 file 2).

162 Data analysis of egg counts was done using the open source software package R version 3.0.2 [34]
163 with the “`glmmadmb()`” function from the `glmmADMB` package [35,36].

164 **Results**

165 *Aedes albopictus* activity period

166 Newly laid *Ae. albopictus* eggs were found only during or after calendar week 22 in mid-May (i.e.
167 the ovitraps were set in week 20), while eggs were still collected up to the calendar week 48 in mid-
168 November when day length was 10 hours and the mean temperature was 7.6 °C. During mild falls,
169 when the mean temperature was still around 9 °C, females continued to lay non-diapausing eggs

170 and fourth instar larvae were found in mid-November. Maximum egg numbers were generally
171 recorded in August in calendar week 34 (Figure 2) when the mean temperature was 21.1 °C. Eggs
172 left outdoors remained in diapause over the winter until mid- to end-April (i.e. calendar week 16 or
173 18) when day length was 11 to 11.5 hours and the mean temperature was 12.3 °C. In summary, *Ae.*
174 *albopictus* reached its activity peak in August, while the eggs went into diapause from mid-
175 November to mid-April.

176 **Temporal and local distribution of *Aedes albopictus***

177 A general overview on *Ae. albopictus* occurrence during the year is shown in Figure 1, whereas
178 detailed maps in Additional file 3 describe the occurrence, establishment and overwintering of the
179 mosquito year by year. In 2003, four out of 166 slats (4.2%) were positive in Mendrisio and
180 Locarno districts (Figure 1, Additional files 3 and 4). Over the following years a steady increase in
181 the number of positive ovitraps was observed till 2007, when *Ae. albopictus* seemed to have
182 established the first seasonally stable population in the border town Chiasso and later also in the
183 neighbouring villages (Mendrisio district). While Mendrisio was the first widely infested district,
184 the presence of the mosquito in the remaining districts was still patchy and of transient nature at the
185 time (Figure 1, Additional file 3). The next turning point took place in 2008 (Figure 1, Additional
186 files 3 and 4) when *Ae. albopictus* was repeatedly observed in residential areas in the Lugano and
187 Locarno districts. Between 2009 and 2011, the mosquito continuously extended its range (Figure 1
188 and Additional file 3) and in 2012 its presence significantly increased in many locations across the
189 canton (Figure 1, Additional files 3 and 4). In 2009, the first overwintering populations were
190 observed at the border region in Mendrisio district and in 2011 in the Lugano district (Additional
191 file 3). During the last two study years, changes were less dramatic, although the trend of *Ae.*
192 *albopictus* expansion continued in Ticino.

193 The detailed local dynamic of how *Ae. albopictus* spread and established in various urban areas
194 across Ticino is shown in Figures 3-6. Service stations and parking areas along the motorway

195 appeared to be one of the first introduction points in the territory (Figures 3, 4 and 6). They were
196 constantly infested by *Ae. albopictus*. In these areas the mosquito clearly managed to establish
197 before its establishment in other urban settlements.

198 In 2003, two of the first positive slats appeared at the first E35 motorway service area after the
199 border from Italy in the Mendrisio district (Coldrerio East) (Figures 1 and 3). The border control
200 area in Chiasso in the south-eastern part of Mendrisio district was also one of the first points of *Ae.*
201 *albopictus* detection. Furthermore traps positioned in the area close to the motorway exit of
202 Mendrisio were positive before other traps in the same area. In this district, the established
203 mosquito population continued to increase over time (Figures 1 and 3).

204 In the Lugano district the first signs of establishment of *Ae. albopictus* appeared in residential areas
205 close to a motorway parking place in 2009 (Figure 4). In the rest of the district the occurrence of the
206 mosquito increased constantly and in 2010 and 2011 some quarters of Lugano, the largest city of
207 the Canton, were infested (Figure 1 and Additional file 3). Similarly, in the Locarno district there
208 was a progressive mosquito occurrence, with the first signs of establishment and overwintering in
209 2011 (Figure 5). Interestingly, two of the first positive slats in 2003 were detected at the Locarno
210 Airport (Locarno district), and again during summer 2004, but could not be detected during the
211 following survey seasons thanks to repeated application of *Bti* and permethrin. The mosquito was
212 then detected again in 2008 and 2009 in camping sites closed to this area.

213 In the Bellinzona district, the mosquito appeared in residential areas in 2009 and then constantly
214 increased, even if its presence remained lower than in the other districts. *Ae. albopictus* established
215 in the North of the district only in 2012 (Figure 1 and Additional file 3). Further north, in the
216 municipality of Biasca (Riviera district), the tiger mosquito appeared repeatedly through the years,
217 but never managed to establish (Figure 1 and Additional file 3).

218 Interestingly, industrial areas were apparently colonised by the mosquito before residential areas
219 with a lead time of two to three years as observed in Agno, Bioggio and Manno municipalities in
220 the Lugano district (Figure 4) as well as in Stabio municipality in the Mendrisio district at the

221 southwest border with Italy (Figure 3). However, from then on the mosquito was more present in
222 residential than in industrial areas (Figures 3 and 4).

223 When ovitraps were placed at altitudes above 400 m, establishment was delayed (Figure 3).

224 In summary, since 2003 *Ae. albopictus* has spread across Ticino from South to North mainly along
225 the trans-European motorway E35. We observed a massive surge around 2011 and 2012 and *Ae.*
226 *albopictus* still continues its spread.

227 **Weather conditions during the annual surveys**

228 Consistent data from the eight weather stations were available between 2006 and 2014. Seasonal
229 and annual mean temperatures recorded at each weather station are summarized in Additional file 2.
230 The annual mean temperature in the study area was 12.5 °C with a mean summer temperature of
231 19.5 °C during the surveys between May and September. July was the warmest month, with a mean
232 temperature of 22 °C with minimum temperatures ranging from 8.5 °C to 11.1 °C and maximum
233 temperatures ranging from 33.7 °C to 38.5 °C. January was the coldest month with a mean
234 temperature of 2.9 °C, with minimum temperatures ranging from -10.7 °C to 14.5 °C and maximum
235 from -3.0 °C to 24.8 °C. Mean among the weather stations of total annual rainfall was 1,789 mm,
236 whereas mean of total precipitation during the survey season was 857 mm. The wettest mosquito
237 season (May-September) occurred in 2008 with a total rainfall of 8,856 mm and the driest occurred
238 in 2013 with 5,816 mm of cumulated rain. The total number of days with minimum temperature
239 below -10 °C, that is the minimum temperature considered for the survival of diapausing eggs
240 [37,38], between 2006-2014 varied from 0 to 14 (Additional file 2).

241 **Relative egg densities**

242 As there were only very few trap locations consistently present since 2003 only data from ovitraps
243 set between 2006 and 2014 were included in the relative egg density analysis. Forty-six traps were
244 always present between 2006 and 2014 (Additional file 5). In these 46 traps a total of 3,358 egg
245 collections were made. Due to lost or damaged traps 370 records had to be removed from the

246 analysis. From the analysed slats we found that the frequency of zero counts decreased as of 2009,
247 while most of the trap counts were still zeros in 2006 (Figure 7). From 2009, increasingly larger
248 counts were recorded across the 46 traps. One trap had 844 eggs in 2014. This trend is also reflected
249 in the GLMM model in that the factor “year” shows increasing ratios for the estimates over the
250 years as compared to the year 2006 (Figure 8 and Table 1). Compared to 2006 the relative density
251 in 2014 has increased 87.4 times from an average biweekly egg count of 0.003 eggs per trap to
252 0.262 eggs per trap.

253 As *Ae. albopictus* has a seasonal activity pattern, it is not surprising that egg densities also varied
254 between months with highest numbers found in August (Figure 8 and Table 1).

255 The variable “rain” that was the cumulative precipitation over the week preceding trap replacement
256 had three levels; “low”, “middle” and “high” with 0-104 mm, over 104 to 209 mm and over 209 to
257 314 mm, respectively. The categories were chosen because visual inspection of the data suggested a
258 non-linear relationship between egg numbers and total rainfall (Figure 8).

259 Intriguingly, rain was negatively associated with the presence of eggs in the traps (Table 1 and
260 Figure 8). This means that egg numbers were higher when the week preceding trap replacement was
261 drier. In contrast to cumulative precipitation, the model did not improve by including temperature as
262 an explanatory variable.

263 For the sake of comparability with other related studies, egg numbers are also presented as mean
264 number of eggs over a period of four consecutive weeks, split by districts (Figure 2). For this
265 descriptive analysis, data from all traps were included. In agreement with the analysis above, there
266 is a general trend in increasing relative egg densities over the years with a sharp increase in egg
267 densities in 2012 compared to the preceding years. Mean densities also reflect the spatial gradient
268 from South to North with highest densities in the district of Mendrisio and the lowest ones in the
269 districts of Bellinzona and Riviera.

270 **Discussion**

271 Since its first detection in 2003 at Ticino's southern tip to Italy *Ae. albopictus* has continuously
272 spread north across the lower valleys, mainly along the trans-European motorway E35. This trend
273 continues as witnessed both by a growing infested area, with a clear South-North gradient in the
274 introduction, spread and establishment of the mosquito.

275 *Ae. albopictus* arrival in Canton Ticino was not so surprising, given the rapid spread that had
276 previously occurred in most regions in North and Central Italy [18,19,39] and the intense road
277 traffic through the trans-European motorway E35 coming from Italy, the most heavily infested
278 country in Europe [21]. On the E35 alone, an estimated daily average of 66,200 vehicles cross the
279 border between Italy and Ticino with over 1 million lorries in 2014 [40]. *Ae. albopictus* introduction
280 with used tyres is considered one of the main pathways globally [41], yet is unlikely to be relevant
281 for Ticino because, to our knowledge, used tyres are not imported into Switzerland. The main
282 pathway of importation is most probably the motorway as the parking areas were the first to be
283 positive for the mosquito and the observed pattern that started the adjacent residential sites have
284 been colonised from there. Industrial areas nearby, where goods from abroad are imported and
285 where many cross-border commuters work, are also likely to be key ports of passive mosquito
286 introductions. From there the mosquito probably might have spread into neighbouring residential
287 areas where *Ae. albopictus* has presumably managed to establish as, perhaps, many breeding sites
288 are available in residential areas and the heating of buildings might create a more suitable
289 microclimate.

290 Due to the heavy traffic crossing the Swiss-Italian border, re-infestation is likely to continuously
291 taking place in addition to the already established local *Ae. albopictus* populations. Once
292 established, it is conceivable that local road traffic fuelled further dispersal of the mosquito, which,
293 for example, could explain the rapid extension of *Ae. albopictus* in 2012. In fact between 2011 and
294 2012 no significant climatic changes occurred as far as we are aware. Even if this mosquito species

295 seems not to fly long distances as field studies suggest [14], the mosquito will still actively disperse
296 albeit at slower speed. In that sense, higher mosquito densities increase the chance of the mosquito
297 spreading into adjacent areas. In contrast, when a built area is isolated, as it was the case for
298 Locarno airport, it might be more difficult for a mosquito to gain ground in residential areas and it
299 is also more easily intercepted by control measures. In 2008 and 2009 when the mosquito
300 reappeared around the airport it was probably linked to touristic activities.

301 Given Ticino shows the climatic conditions for an establishment of *Ae. albopictus* [42], it is not
302 surprising to see a similar trend as in other areas in Europe south of the Alps, mainly along the
303 Mediterranean coast [43,44], and in comparable areas in the USA [15]. Generally, the annual mean
304 temperature of 12.5 °C recorded in the study area exceeds the suggested 11°C threshold for *Ae.*
305 *albopictus* development [45]. Similarly, a mean temperature of 2.9 °C in January, which is above
306 the suggested thresholds of 0 °C [45], would also not prevent eggs from overwintering. Mean
307 precipitation of 1,789 mm during the whole year and 857 mm during the mosquito season between
308 May and September also provide sufficient breeding sites [46]. However, a closer look at the local
309 weather conditions suggests an impact on the speed of how *Ae. albopictus* infests new areas. In
310 areas with an annual mean of 12 °C, including Biasca (Riviera district), Magadino/Cadenazzo and
311 Stabio (Locarno and Mendrisio districts, respectively), the spread and following establishment were
312 delayed. Here, the minimum mean January temperatures varied between 1.5 °C and 2.2 °C. At
313 Magadino/Cadenazzo (Locarno district) and Stabio (Mendrisio district) the minimum temperatures
314 were frequently even below -10 °C. Minus 10 °C is considered the absolute minimum temperature
315 for the survival of overwintering eggs [37,38]. This may explain why the mosquito's spread is slow
316 or absent in those regions.

317 In Biasca (Riviera district), the northernmost surveyed area, *Ae. albopictus* was repeatedly detected
318 at the end of the survey season (August- September) in several years, yet there are no signs of firm
319 establishment even though weather conditions are comparable to Magadino/Cadenazzo and Stabio

320 where the mosquito is, indeed, established. A possible explanation could be that there is less
321 importation of adults in vehicles.

322 The survey focused on areas below 400 m a.s.l. Regions at higher altitudes were included if *Ae.*
323 *albopictus* was spreading into the neighbouring valley floors [27]. The mosquito was observed there
324 even though it has difficulty to establish, probably because of unfavourable weather conditions for
325 establishment and low road traffic.

326 Even though estimation of establishment and overwintering capacity using ovitrap data is not
327 optimal because of the competition with existing breeding sites, our detailed analysis showed a
328 clear dynamic trend. *Ae. albopictus* appeared sporadically in places and then became more and
329 more present in the same spot the following years, suggesting gradual establishment of locally
330 reproducing populations that manage to overwinter. However, our observations do not allow an
331 estimate of the proportion of the *Ae. albopictus* population in Ticino that is stable and what
332 proportion is continuously displaced passively. A better understanding of the population dynamics
333 and a better knowledge of the threshold required for the establishment of a population are needed to
334 assess the potential for further establishment and to improve targeted mosquito surveillance.

335 Nevertheless, considering that low temperatures reduce mosquito establishment, whereas intense
336 road traffic and habitat suitability of the residential areas appear to favour the mosquitoes arrival
337 and establishment, our observations help in setting priorities in the survey and control measures.
338 Therefore, we suggest prioritising residential areas as well as areas where traffic and human
339 population densities are higher.

340 The onset of seasonal activity of *Ae. albopictus* occurred in mid- to end-April with the diapause
341 exit, when day length and mean temperature were 11-11.5 hours and 12.3 °C, respectively. This
342 corresponds to what was reported from Rome (11 to 11.5 hours daylight with mean temperatures of
343 10 to 11 °C) [47], where the maximum egg counts were observed in August, when the mean
344 temperature was about 21.1 °C. This corresponds to what was reported in other Italian regions such
345 as Emilia-Romagna [48]. In Ticino, *Ae. albopictus* was active until mid-November when day length

346 corresponded to 10 h of daylight and when the mean temperature was about 8 °C. To date, no
347 winter activity has been observed, in contrast to what was reported from slightly warmer regions
348 such as Emilia-Romagna and Rome with an annual mean temperature of 12.5 °C [49] and 15.7 °C
349 [50], respectively. There, freshly deposited eggs and adults were also reported during winter
350 [32,48]. The trend of incessant and prolonged diapause with higher latitude is a phenomenon also
351 observed in northern America [51]. Generally, the pattern observed here corresponds to the Italian
352 Province of Trento, another sub-Alpine area where *Ae. albopictus* is overwintering as dormant eggs
353 and where climatic conditions are similar to Ticino [52].

354 To detect *Ae. albopictus* and estimate its relative population size, ovitraps were the method of
355 choice because these traps are sensitive at low mosquito densities [3], relatively cheap and require
356 little maintenance. Some authors have, however, raised concerns over the validity of ovitraps for
357 relative density estimates due to their competing nature with existing breeding sites (e.g. [53]) and
358 because a single female may deposit its eggs in multiple sites [54], a behaviour known as skip
359 oviposition. The use of indices and overall mean values of non-normally distributed egg counts
360 rather than working directly with the actual data as done in the present study may explain the
361 different conclusions among studies. Intriguingly, in support of our study, Carrieri *et al.* [55,56]
362 found that egg counts, estimated by means of ovitrap monitoring, were a reliable proxy for the mean
363 number of biting females per unit area as well as larval productivity. In an attempt to learn more
364 about the dynamics of relative mosquito population densities we therefore decided to include in our
365 analysis also actual egg counts rather than reducing the whole data set to a mere presence-absence
366 table. Considering the highly over dispersed data set owing to high numbers of empty traps and to
367 account for correlation due to repeated measures in the same trap over time we have chosen to
368 model the data with a GLMM with a negative binomial link function. Initially we had also looked at
369 a model with a zero inflated negative binomial distribution, but we did not see an improvement in
370 fitting the data.

371 We also found that more eggs were laid when weekly accumulated rainfall was lower. On the one
372 hand, this may be explained by breeding sites becoming less frequent during drier periods making
373 the ovitraps more attractive to egg laying females, so that egg counts are an overestimate of relative
374 abundance. On the other hand, intense precipitation reduces abundance over short periods of host
375 seeking females [57]. In line with the latter, it may be argued that the artificial containers in which
376 *Ae. albopictus* is mainly breeding are still present during dry periods because under these
377 circumstances residents still water flowers or store water in tanks in their gardens.

378 The observations made in the current study together with the overall trend in Europe [21] as well as
379 models predicting habitat suitability under present and future climate scenarios [42,58] suggest that
380 *Ae. albopictus* will continue to spread from the Mediterranean regions further into northern Europe
381 by passive transport. Indeed, *Ae. albopictus* is already spotted more frequently along the E35 north
382 of the Alps both in Switzerland [59,60] and Germany [23–25]. *Ae. albopictus* is expected to infest
383 areas that are already climatically suitable such as the region around the Lake Geneva [42], given
384 the area is also well connected through major traffic routes to the South of France, where the
385 mosquito is well established. In addition, although models consider environmental mean
386 temperatures [42,44,46,61], urban settlements offer microclimates that are warmer in winters than
387 the ones recorded by weather stations because of heating. Urban areas could favour the *Ae.*
388 *albopictus* overwintering and allow its establishment in regions not considered suitable so far.

389 In Europe, autochthonous cases of dengue and chikungunya appeared shortly after the mosquito's
390 peak season (late summer to mid autumn) when mosquito densities were high enough and
391 temperatures still favourable for viral replication in the mosquito [6–13]. While egg count is a good
392 indicator of the presence of *Ae. albopictus*, and to some extent also of its relative density, it would
393 be useful if such data could also be used in more detail to make inference of the risk for disease
394 transmission and outbreaks. Carrieri *et al.* [56] estimated epidemiological thresholds, modelling
395 vectorial capacity calibrated by egg numbers against the number of host seeking females using
396 landing catches. Epidemic thresholds should still be estimated with parameters measured in the

397 Canton of Ticino following e.g. the Italian model by including other factors such the number of
398 infected people returning from endemic areas [62–64].

399 **Conclusions**

400 *Ae. albopictus* has firmly established in Ticino and is continuously expanding its range from South
401 to North. Though the local patterns may differ due to variations in traffic load and local climatic
402 conditions, our results suggest a more universal trend in that *Ae. albopictus* continues spreading and
403 increasing in densities, which is a call for continued surveillance.

404 **Competing interests**

405 No-financial competing interests

406 **Authors' contributions**

407 EF, LE, PM and MT conceived the study. EF and LE performed the field and laboratory work. LE
408 managed the data and plotted the maps. PM made the statistical analysis. EF wrote the first draft.
409 EF, LE and PM analysed the data and prepared the manuscript. All authors contributed to the final
410 draft, and have read and approved it.

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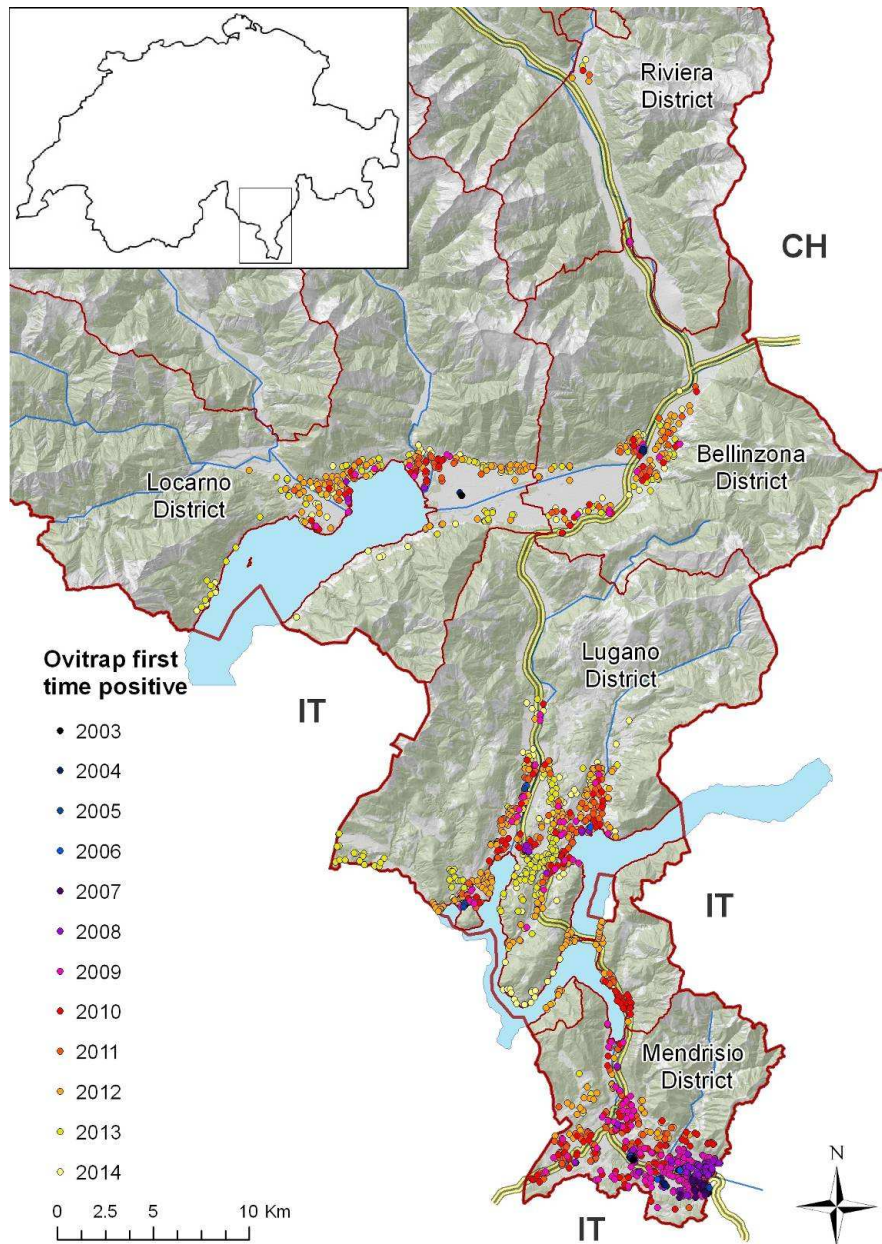
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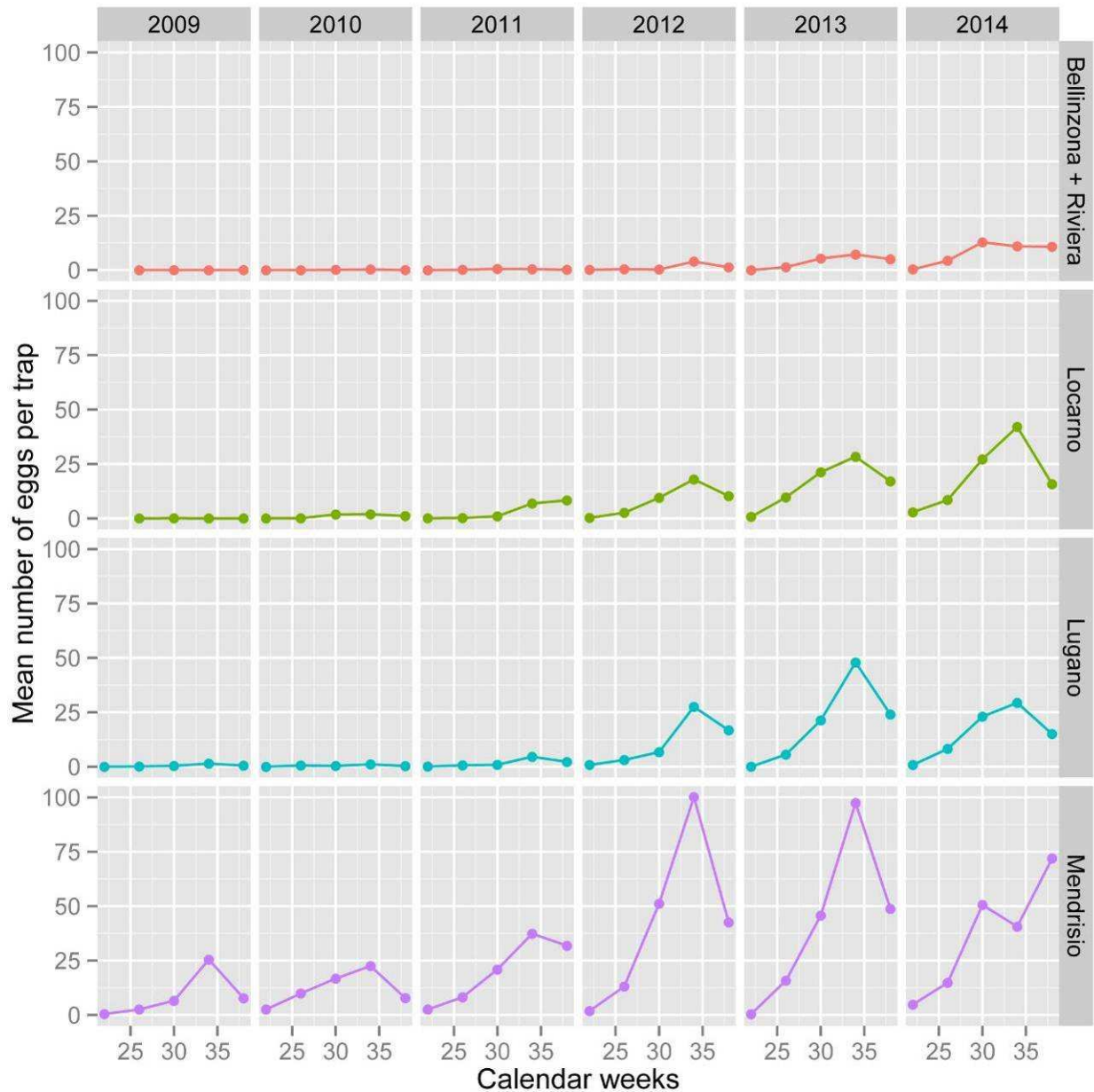
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592



596 **Figure 1 - Spatial and temporal distribution of *Aedes albopictus* in the Canton of Ticino since**
597 **its introduction to Switzerland**

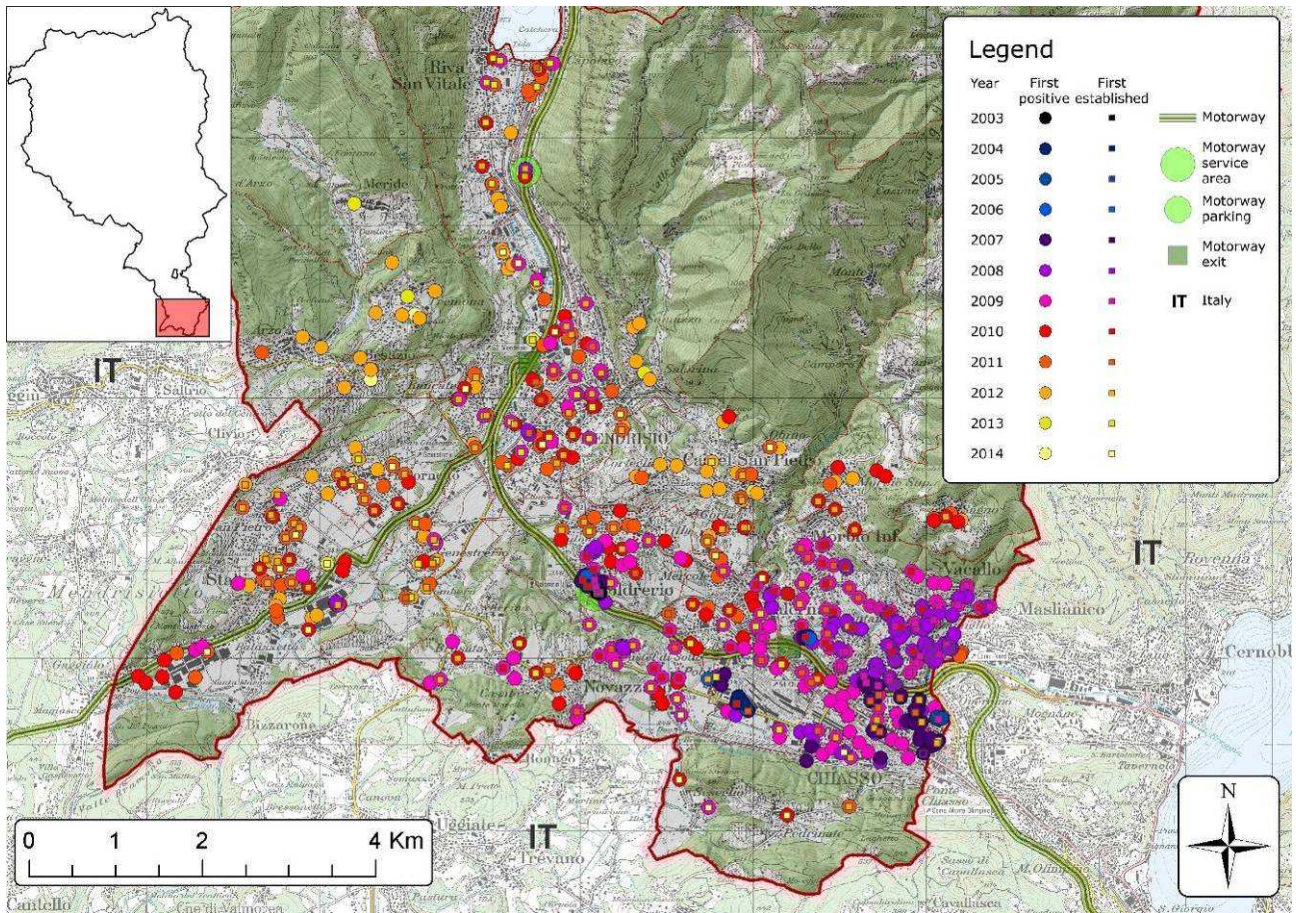
598 Dot colours indicate when the ovitraps were first positive to *Ae. . albopictus*. The yellow lines show
599 the motorways. CH: Switzerland; IT: Italy. Map layers were purchased from the Swiss Federal
600 Office of Topography.



601

602 **Figure 2 – Mean *Aedes albopictus* egg numbers per trap according to districts between**
 603 **2009 and 2014.**

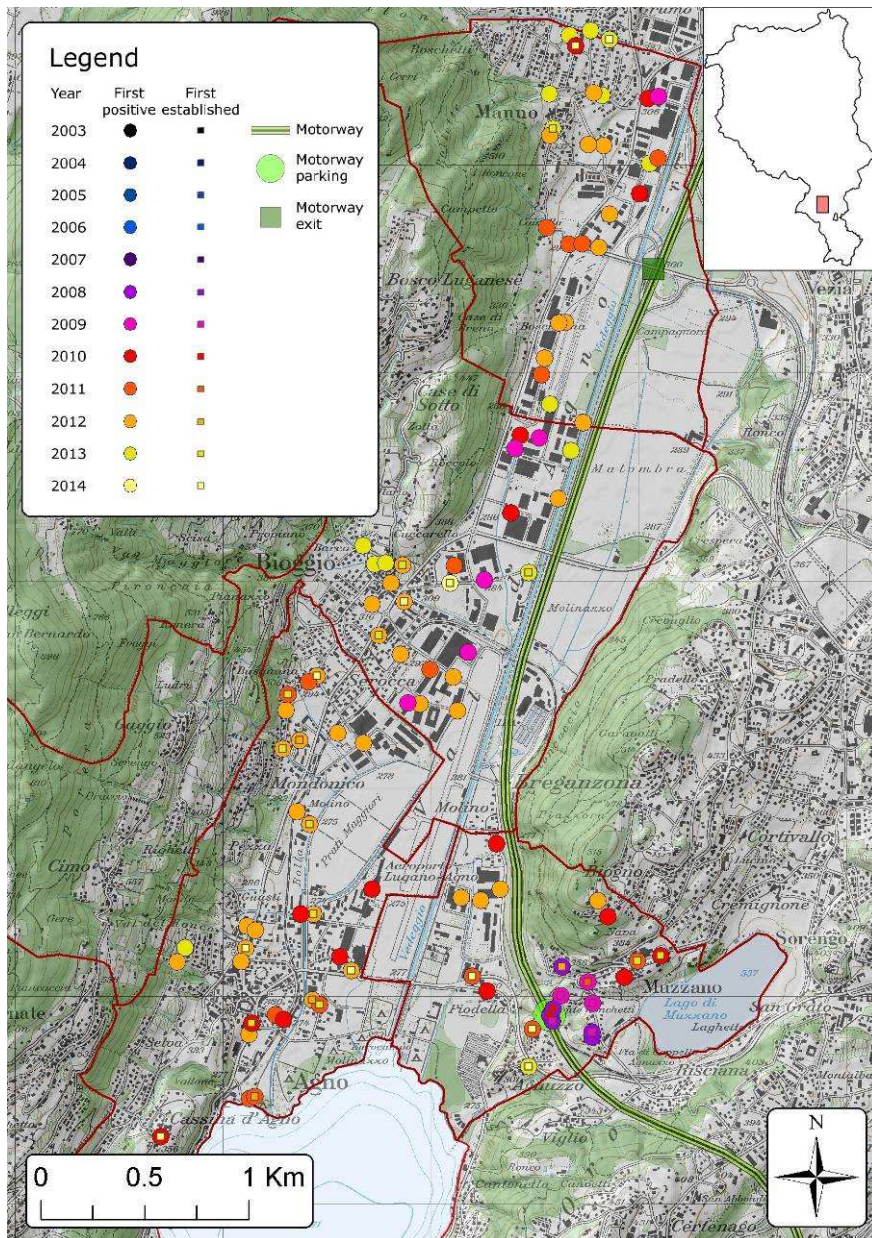
604 The graphs show the average egg numbers per trap by year and calendar week.



605

606 **Figure 3 – Detailed view of *Ae. albopictus* first detections and first evidences of**
 607 **establishment on ovitraps in the Mendrisio district from 2003 to 2014.**

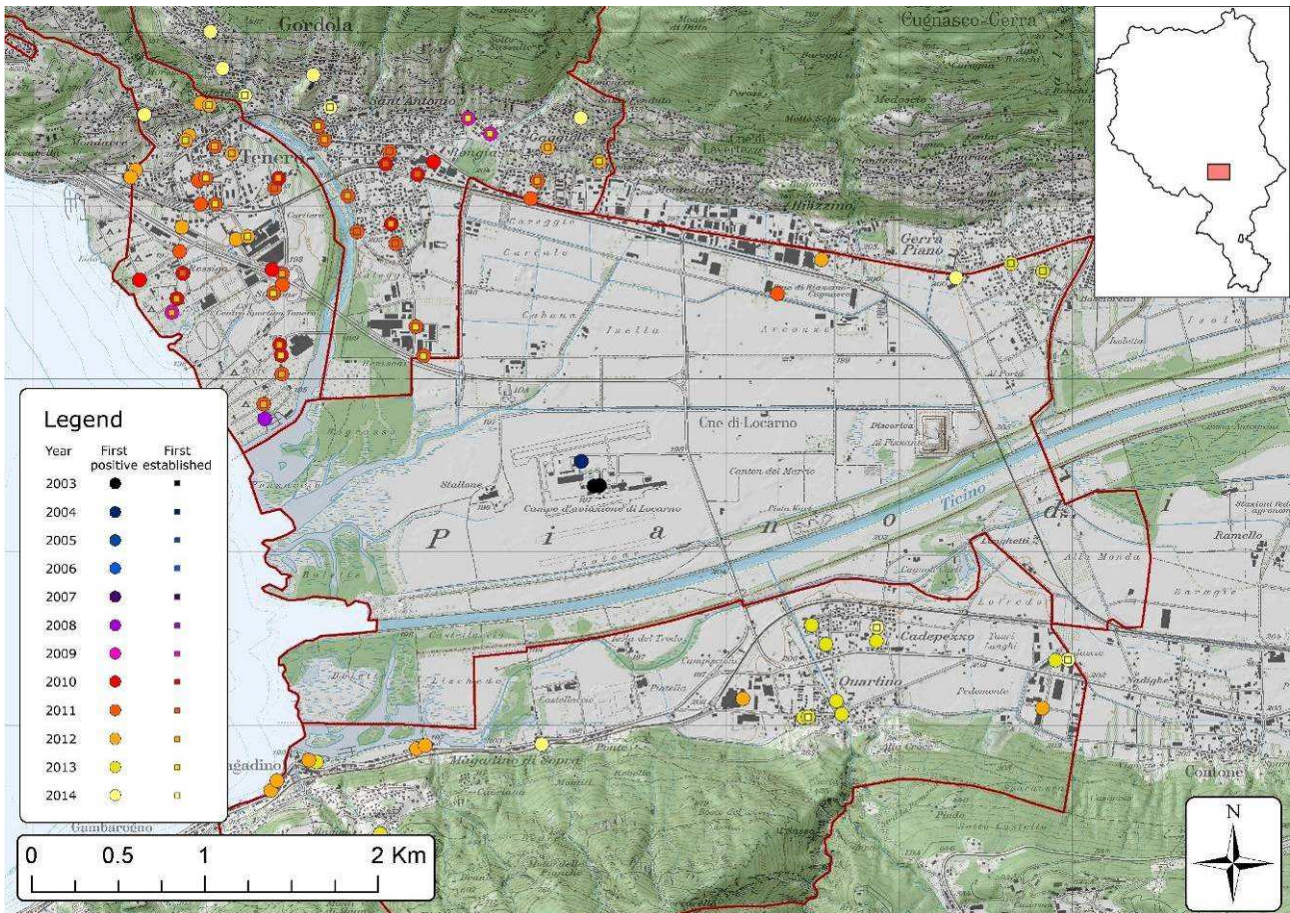
608 Dots represent an ovitrap and the colour indicates the year when ovitraps were positive
 609 for *Ae. albopictus* the first time. The squares indicate the year in which *Ae. albopictus* was
 610 considered to have established a local population. For this an ovitrap had to be positive for
 611 three consecutive four week periods. Map layers were purchased from the Swiss Federal
 612 Office of Topography.



613

614 **Figure 4 – Detailed view of *Ae. albopictus* first detections and first evidences of**
 615 **establishment on ovitraps in the Lugano district from 2003 to 2014.**

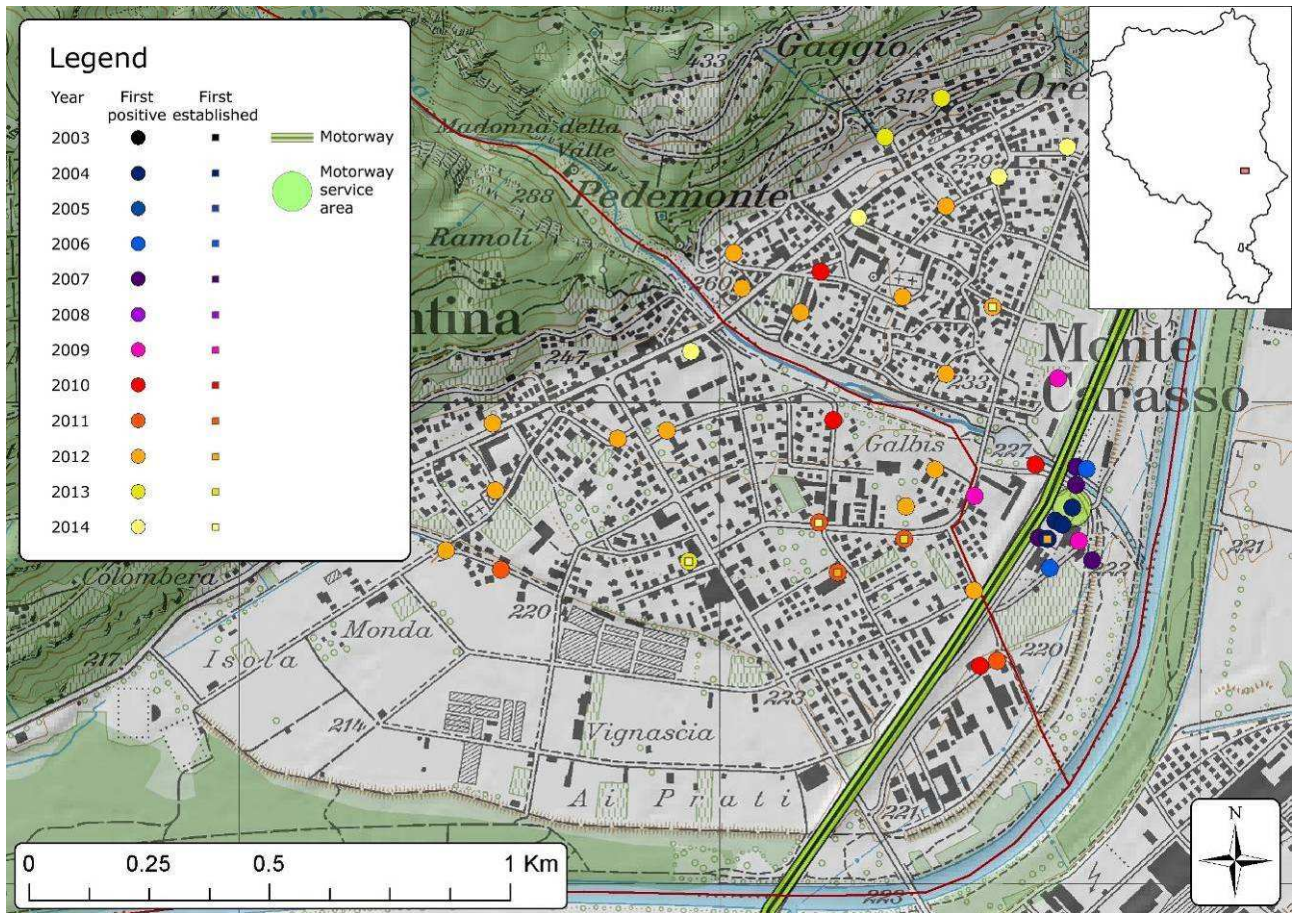
616 Dots represent an ovitrap and the colour indicates the year when ovitraps were positive
 617 for *Ae. albopictus* the first time. The squares indicate the year in which *Ae. albopictus* was
 618 considered to have established a local population. For this an ovitrap had to be positive for
 619 three consecutive four week periods. Map layers were purchased from the Swiss Federal
 620 Office of Topography.



621

622 **Figure 5 – Detailed view of *Ae. albopictus* first detections and first evidences of**
 623 **establishment on ovitraps in the Locarno district from 2003 to 2014.**

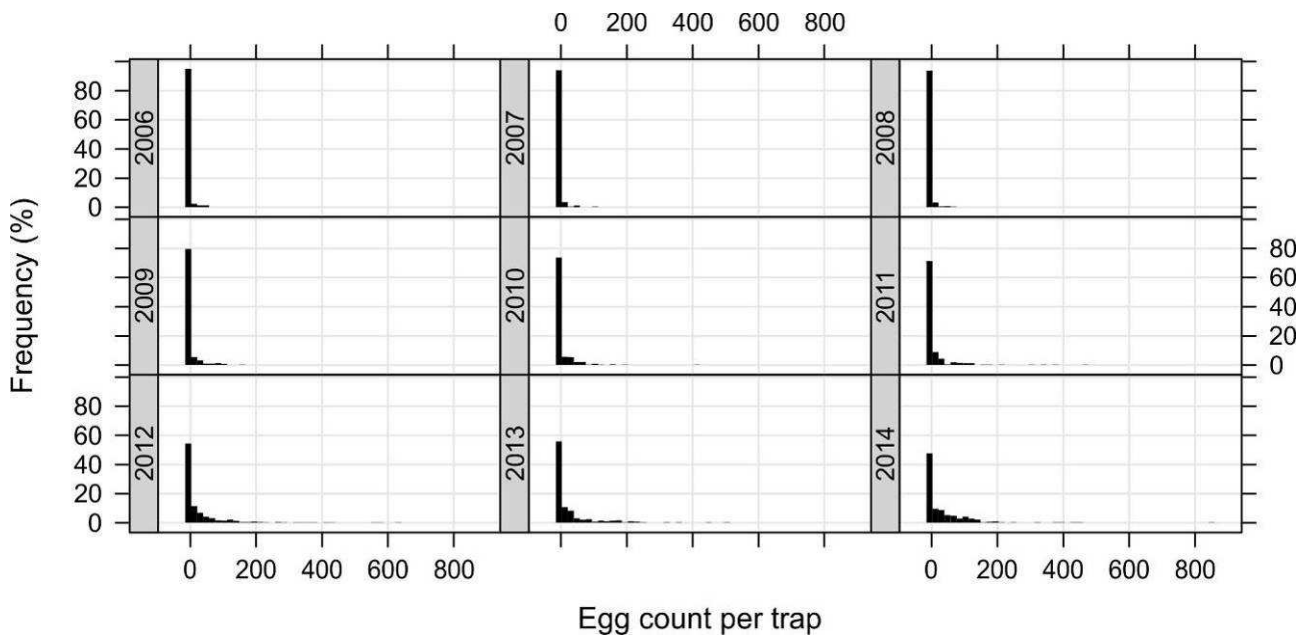
624 Dots represent an ovitrap and the colour indicates the year when ovitraps were positive
 625 for *Ae. albopictus* the first time. The squares indicate the year in which *Ae. albopictus* was
 626 considered to have established a local population. For this an ovitrap had to be positive for
 627 three consecutive four week periods. Map layers were purchased from the Swiss Federal
 628 Office of Topography.



629

630 **Figure 6 – Detailed view of *Ae. albopictus* first detections and first evidences of**
 631 **establishment on ovitraps in the Bellinzona district from 2003 to 2014.**

632 Dots represent an ovitrap and the colour indicates the year when ovitraps were positive
 633 for *Ae. albopictus* the first time. The squares indicate the year in which *Ae. albopictus* was
 634 considered to have established a local population. For this an ovitrap had to be positive for
 635 three consecutive four week periods. Map layers were purchased from the Swiss Federal
 636 Office of Topography.

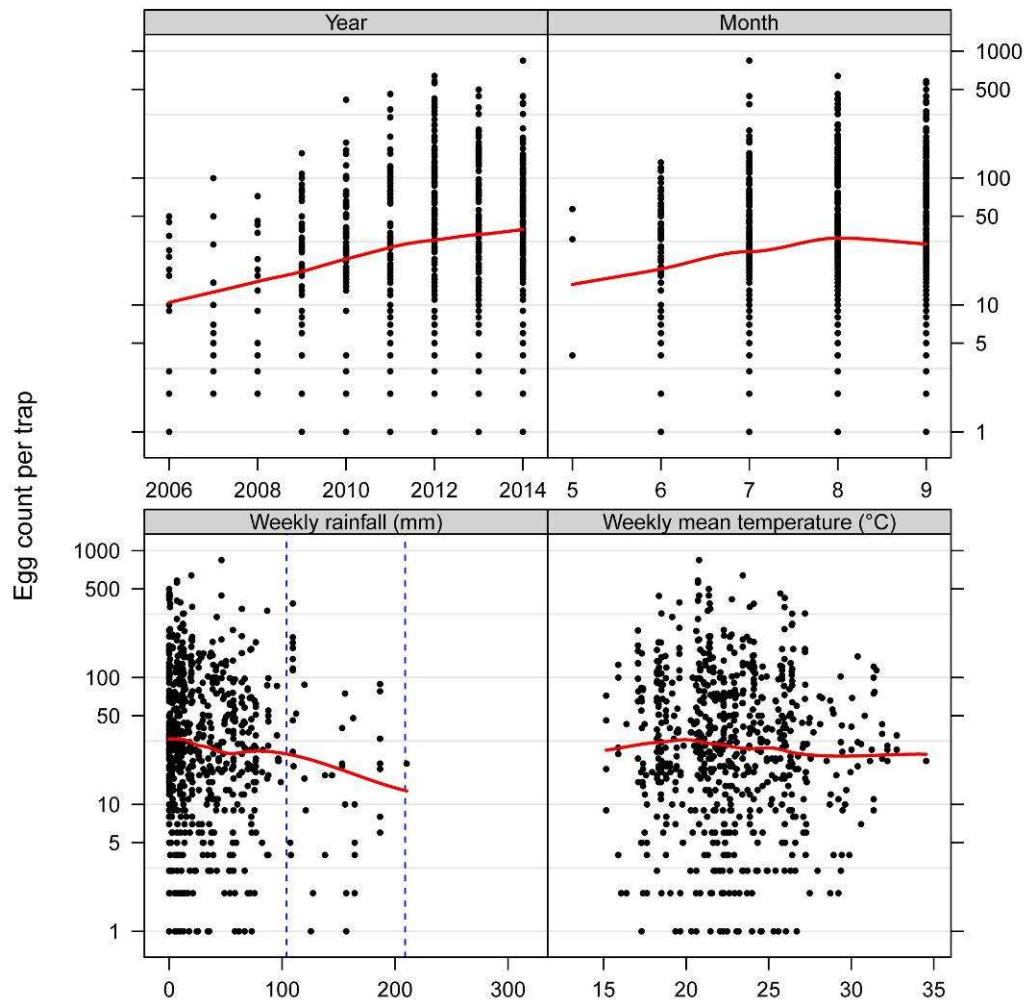


637

638 **Figure 7 - Egg numbers in sentinel sites between 2006 and 2014.**

639 The histograms show the percentage frequencies of egg counts in the 46 sentinel traps

640 present throughout 2006 to 2014.



641

642 **Figure 8 - Relationship between egg numbers and covariates in the sentinel traps of the**
 643 **Ticino *Aedes albopictus* surveillance since 2006**

644 The multi-panel scatterplots show the egg counts on each slat from the 46 sentinel ovitraps
 645 as a function of the four covariates; year, month, rain and temperature. Rain was
 646 calculated as the cumulative precipitation over the week before collecting the slat and
 647 temperature accordingly as the mean temperature during the preceding week. A LOESS
 648 smoother (red line) was added to aid visual interpretation. The visual inspection indicates
 649 an increase in egg counts over the years and a seasonal maximum in August. The variable
 650 “rain” was split into three levels, represented by the vertical blue stippled lines. The levels
 651 were “low”, “middle” and “high” with 0-104 mm, over 104 to 209 mm and over 209 to 314
 652 mm, respectively. Note that egg counts are plotted on a logarithmic scale; and hence zero
 653 counts are not shown due to points at infinity.

654

655 **Table**

656 **Table 1 – Result summary for the negative binomial model for the relationship between**
657 **egg counts, year, month and precipitation for the years 2006 to 2014.**

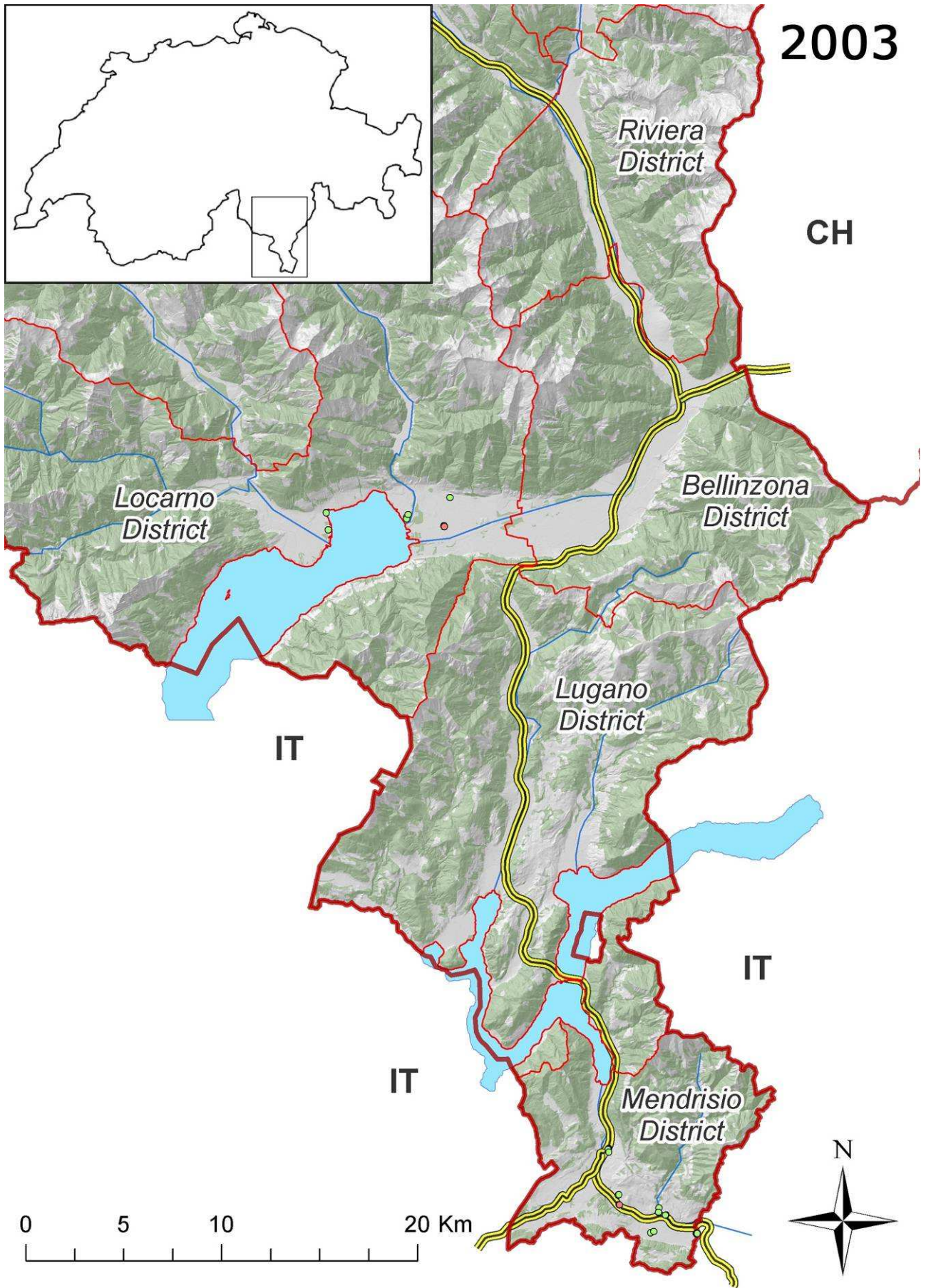
Predictor	Coefficient β (\log_2)	SE(β) (\log_2)	z-value	p-value
Intercept	-5.81	0.596	-9.75	< 0.001
2007	1.278	0.374	3.42	< 0.001
2008	0.292	0.36	0.81	n.s.
2009	1.88	0.341	5.51	< 0.001
2010	2.609	0.369	7.08	< 0.001
2011	2.801	0.345	8.13	< 0.001
2012	4.383	0.355	12.36	< 0.001
2013	3.935	0.366	10.75	< 0.001
2014	4.471	0.358	12.47	< 0.001
June	2.745	0.467	5.88	< 0.001
July	4.402	0.468	9.4	< 0.001
August	5.125	0.464	11.03	< 0.001
September	5.1	0.471	10.83	< 0.001
Rain „middle“	-1.131	0.283	-4	< 0.001
Rain „high“	-2.406	0.906	-2.65	< 0.01

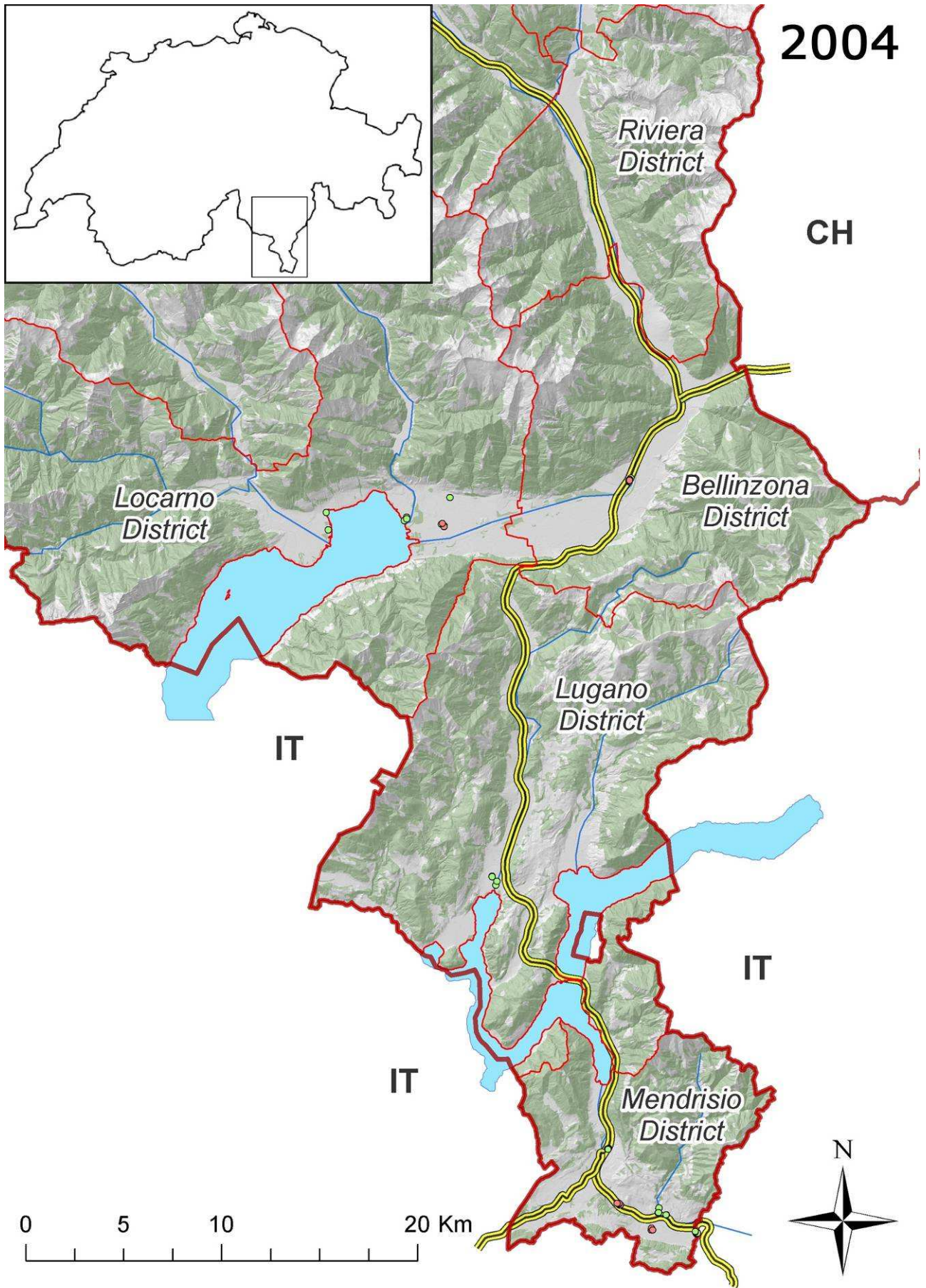
658 Negative binomial dispersion parameter $\alpha = 0.10125$ (SE = 0.0047812). n.s.: not significant.

673

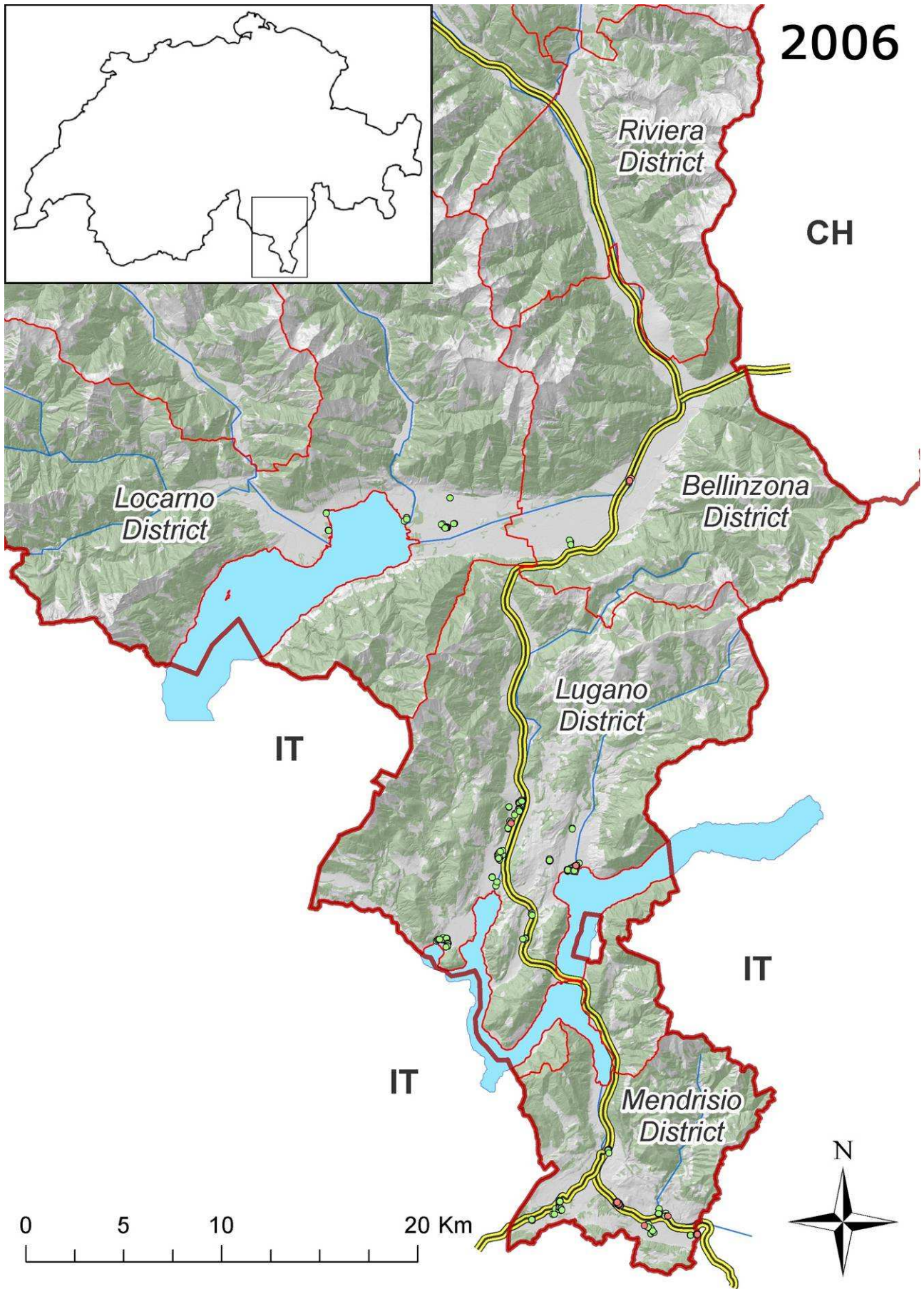
674 **Additional file 3 – *Aedes albopictus* presence in Canton Ticino (southern Switzerland)**
675 **over the years.**

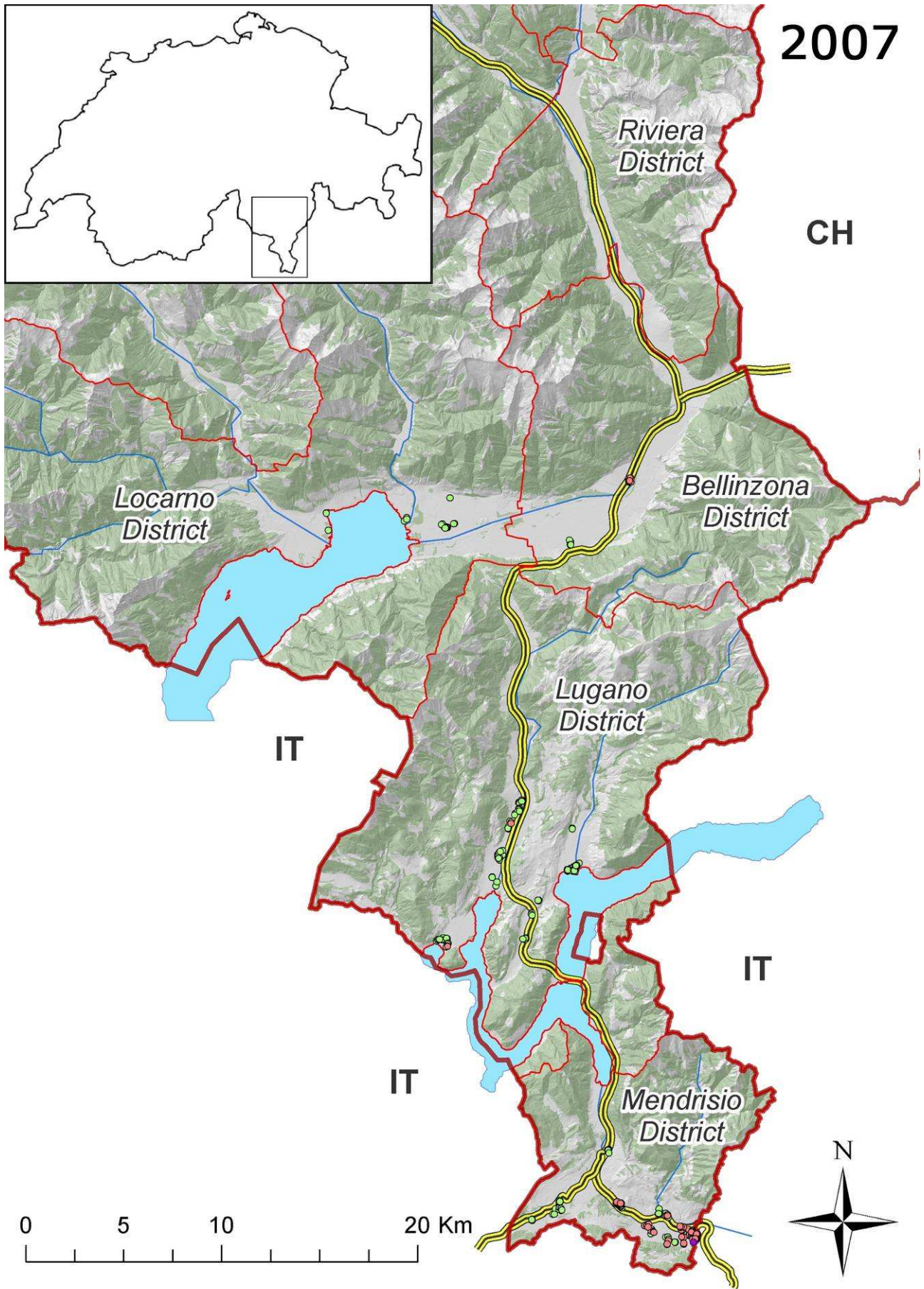
676 The file contains a series of maps for each year from 2003 to 2014. Each map shows for a
677 particular year where ovitraps were positive or negative for *Ae. albopictus* eggs. A dot
678 represents an ovitrap and is colour-coded according to its status; green indicates the trap
679 was always negative, red shows that eggs were found at least once, purple indicates
680 seasonal establishment (i.e. the trap was repeatedly positive over at least three months),
681 and blue indicates the overwintering (i.e. the trap was positive the last control round of a
682 year and the first control round of the following one). Map layers were purchased from
683 the Swiss Federal Office of Topography.



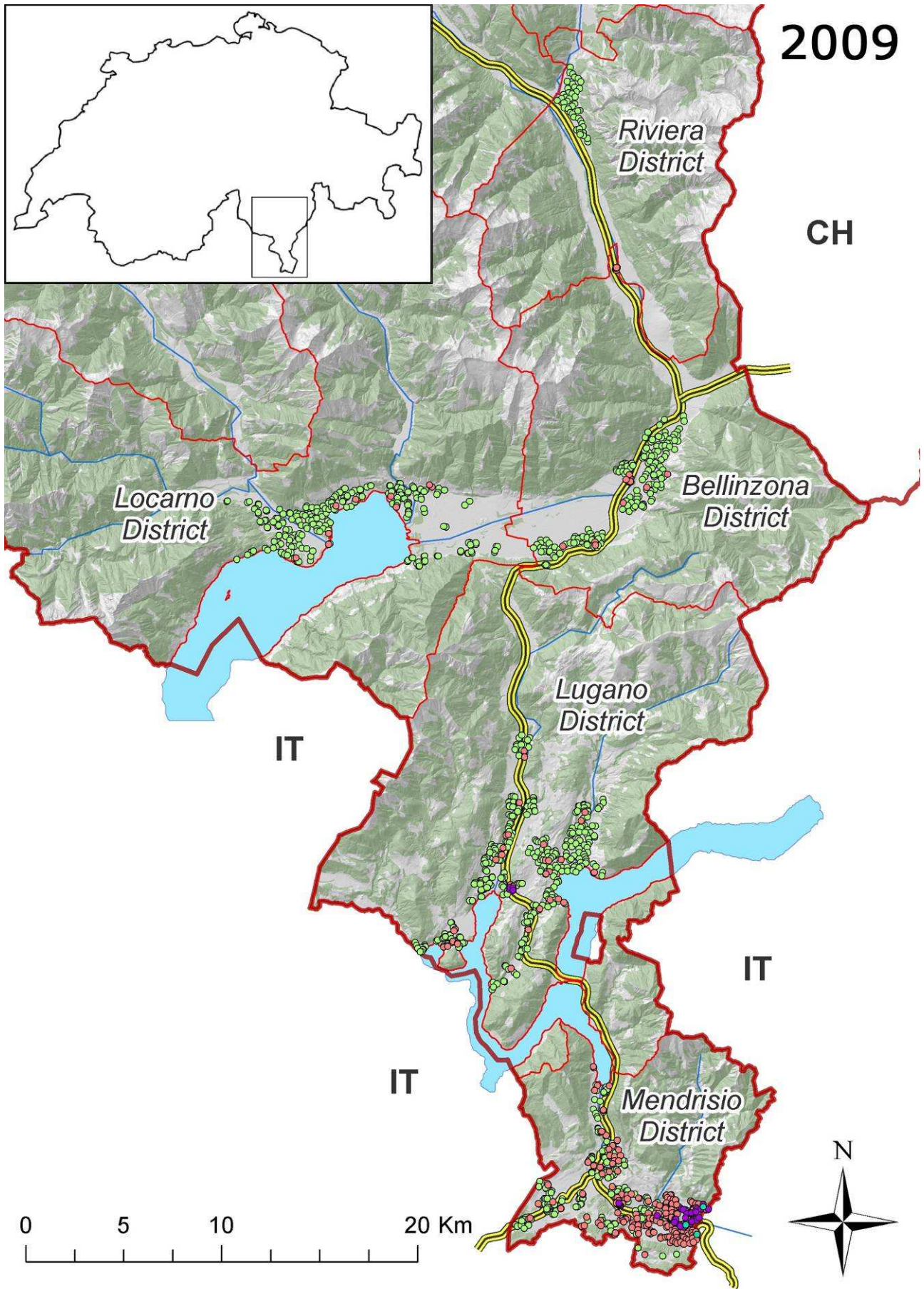


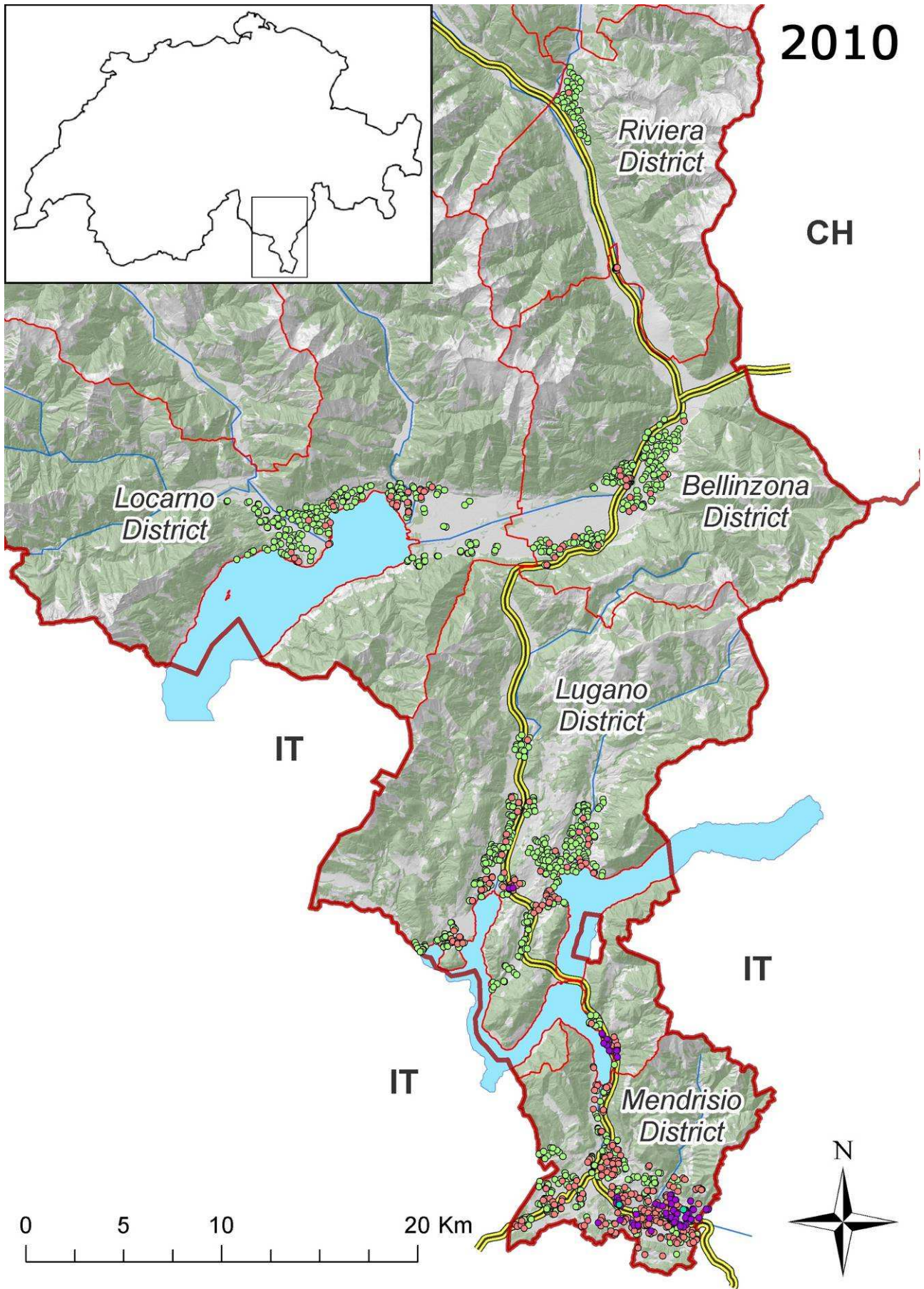


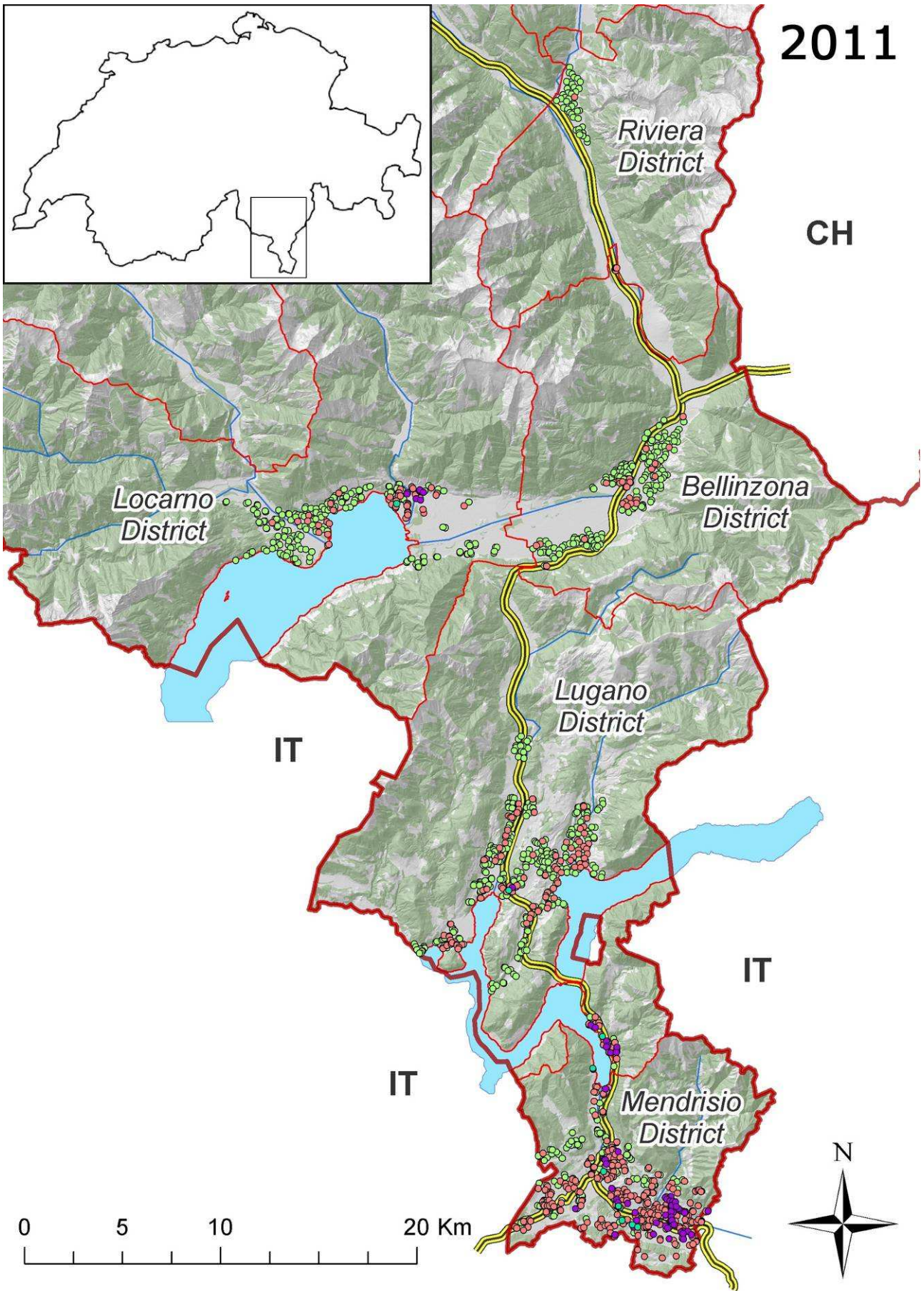


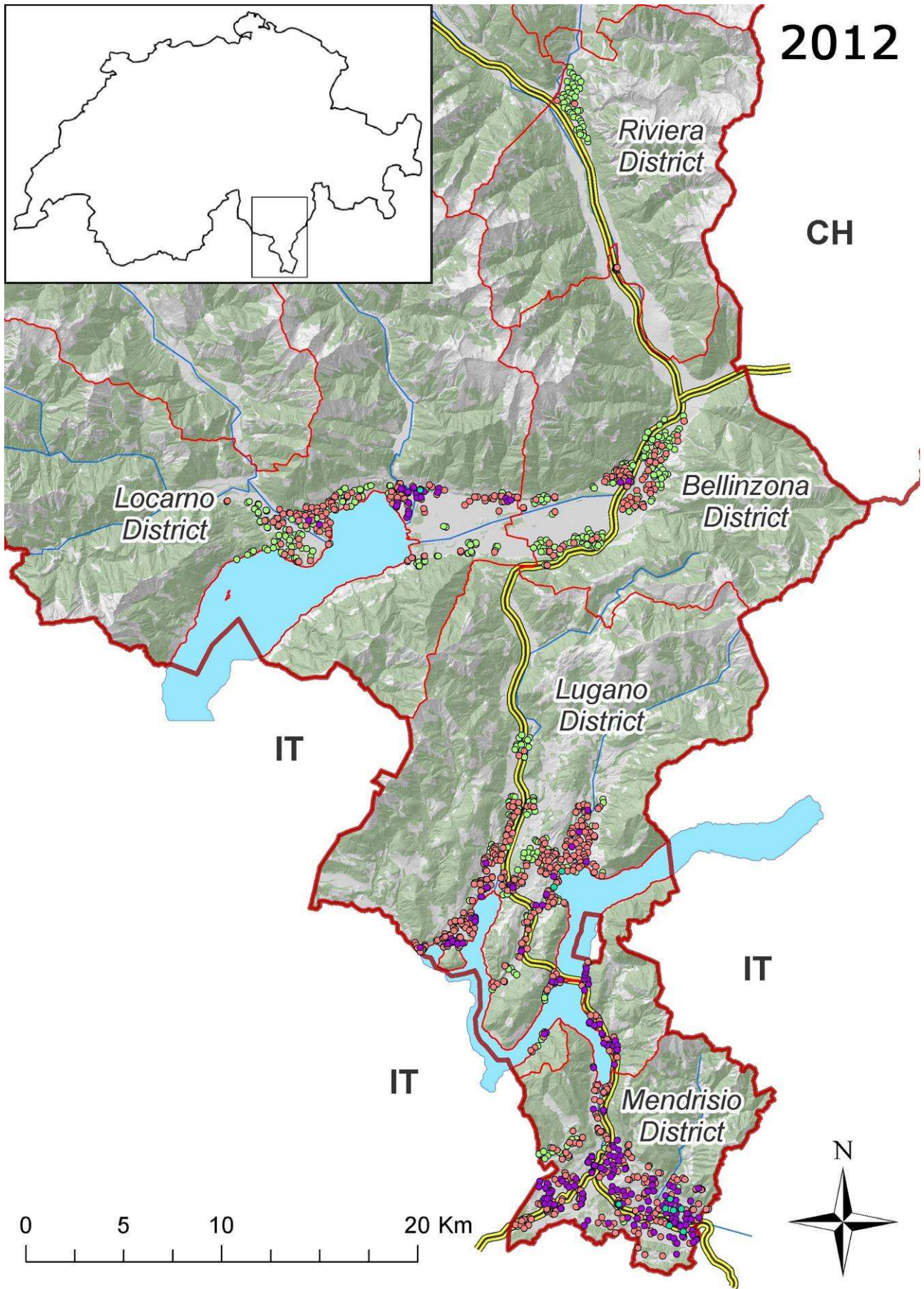


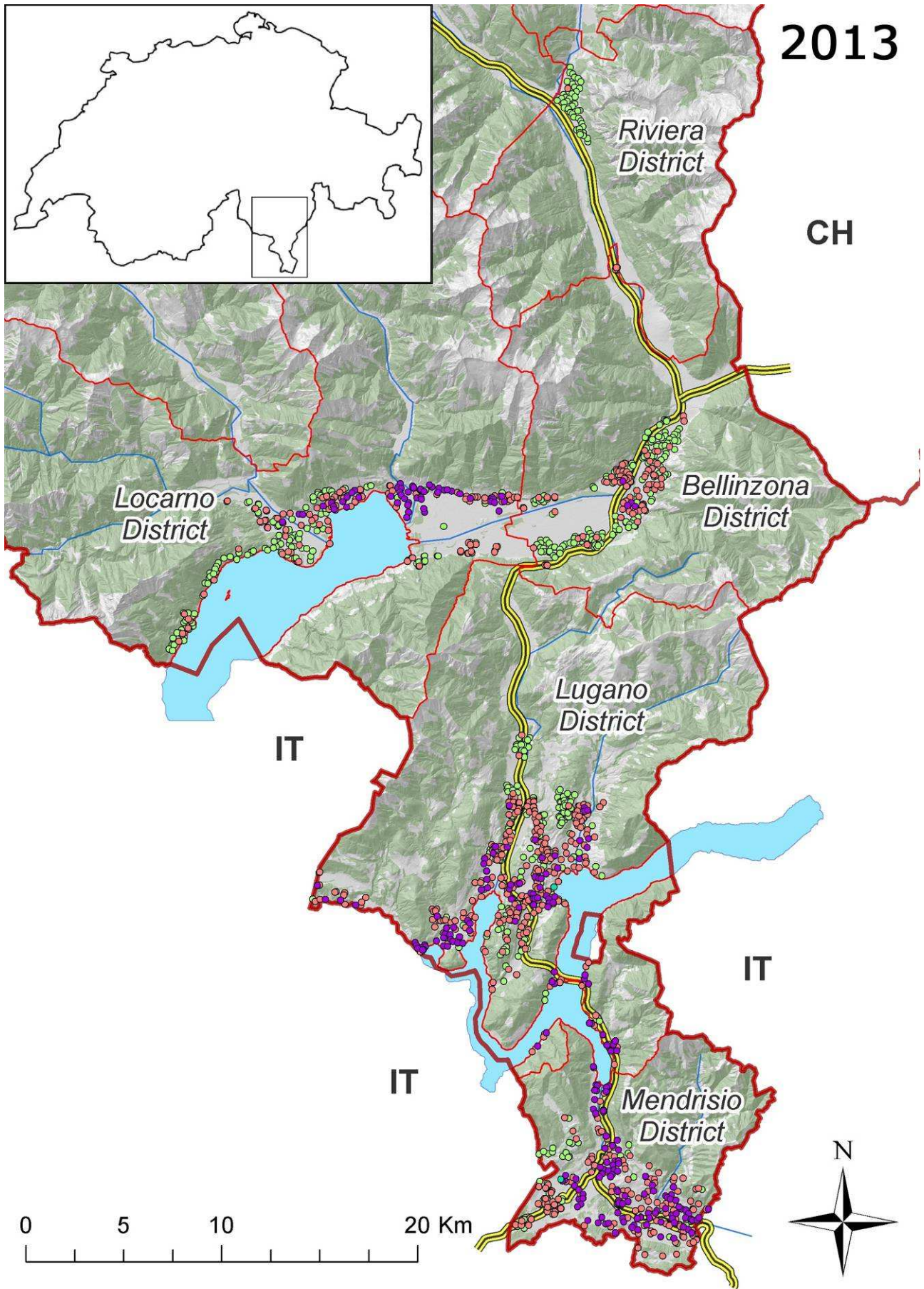


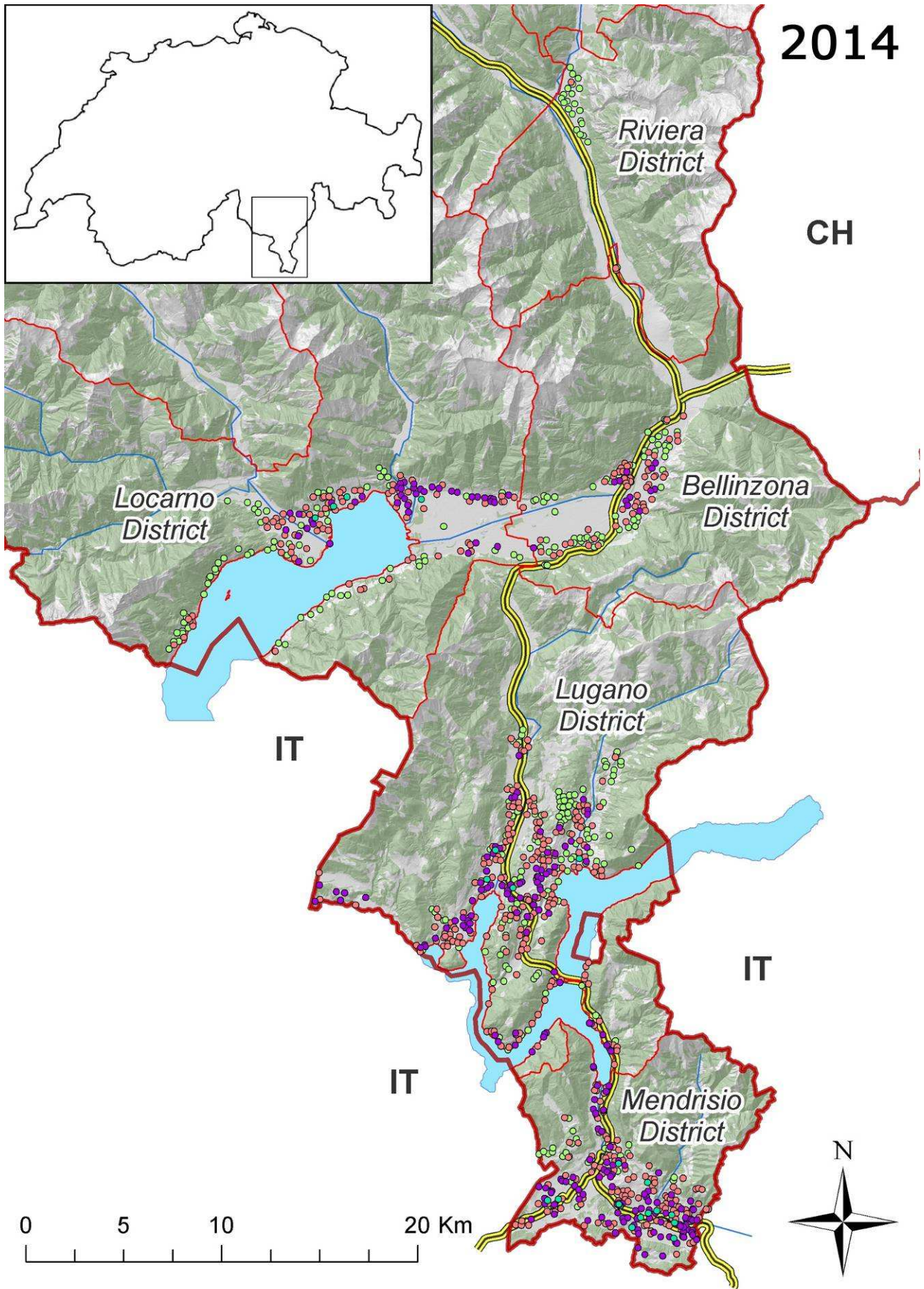












696

697 **Additional file 4 – Result summary of the Ticino surveillance programme from 2003 to**
698 **2014**

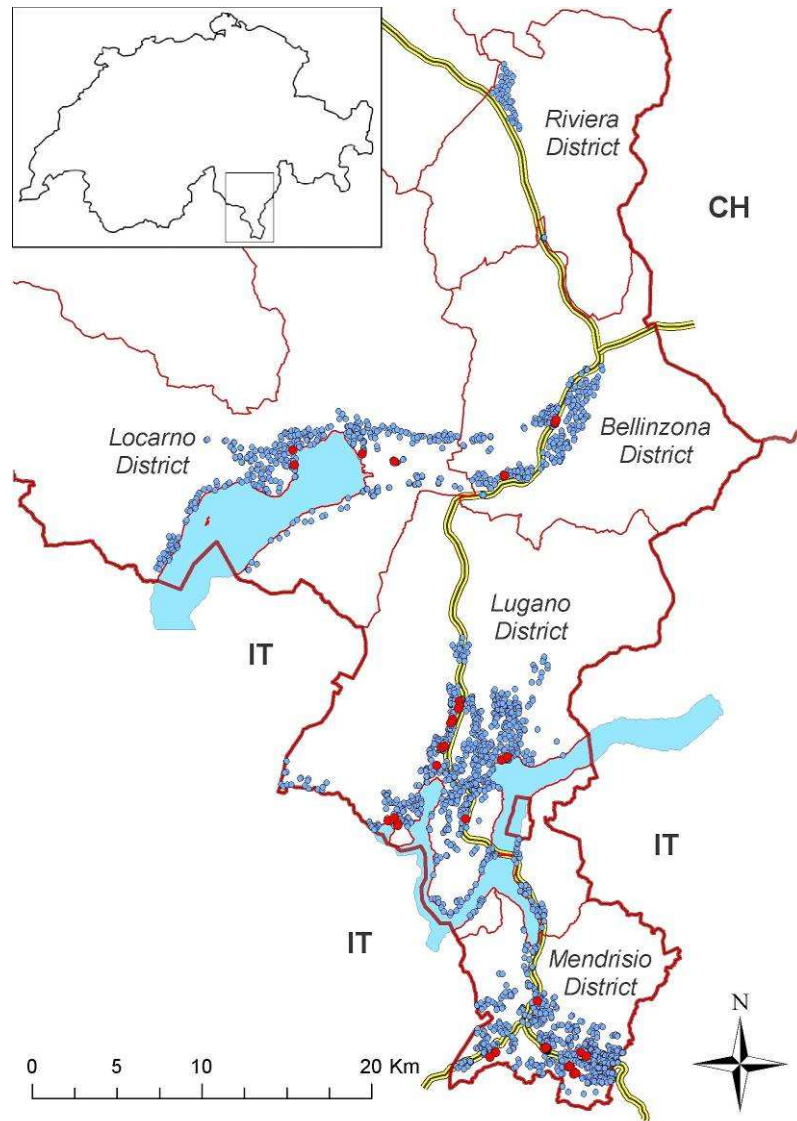
Year	Number of ovitraps	Number of control rounds	Total number of slats	Proportion of positive slats (%)	Area covered by surveillance programme (km2)	Number of traps indicating seasonal establishment	Number of traps indicating overwintering
2003	34	6	166	4.2	0.94	0	0
2004	57	9	465	5	1.06	0	0
2005	189	12	1022	1.5	3.56	0	0
2006	235	10	2028	1.7	3.75	0	0
2007	292	10	2689	5	4.56	1	0
2008	466	14	4919	8.9	7.06	9	0
2009	1241	10	10526	7.3	38.06	37	3
2010	1342	11	13418	7.6	41.75	67	2
2011	1357	10	13305	10.9	41.94	75	11
2012	1361	9	11961	24.2	45.19	206	11
2013	1389	9	7069	29.4	54.69	256	2
2014	1022	9	5464	36.6	60.13	265	23

699

700 Only data from available slats are reported. The area covered by the surveillance
701 programme was estimated by adding the surface area of a virtual 250 m by 250 m grid that
702 covered the ovitraps.

703

704 **Additional file 5 – Geographical distribution of ovitraps in the Canton of Ticino**
705 **between 2003 and 2014.**



706

707 The red dots represent the 46 sentinel ovitraps included in the analysis of the egg counts
708 between 2006 and 2014. The thick and thin red lines indicate the national and district
709 borders, respectively. The yellow line shows the motorways, including the trans-European
710 motorway E35 running from South to North. Light blue shaded areas are lakes. CH:
711 Switzerland; IT: Italy

712

4.2 PAPER 2

Strategies of a Thirteen Year Surveillance Programme on *Aedes albopictus* (*Stegomyia albopicta*) in Southern Switzerland. 2015. *Parasites & Vectors* 8 (1): 208. doi:10.1186/s13071-015-0793-6.

Flacio, Eleonora, Lukas Engeler, Mauro Tonolla, Peter Lüthy, and Nicola Patocchi

Based on data collected during the survey on *Aedes albopictus*, the surveillance system, consisting in survey and control measures, adopted different strategies following the dynamic of the mosquito spread along the Canton Ticino. Aim of the work was to keep mosquito densities at a bearable level for the residents and to reduce the risk of autochthonous transmission of *Ae. albopictus* borne diseases.

This article can be free downloaded from the website of Parasites & Vectors (<http://www.parasitesandvectors.com/>)

RESEARCH

Open Access

Strategies of a thirteen year surveillance programme on *Aedes albopictus* (*Stegomyia albopicta*) in southern Switzerland

Eleonora Flacio^{1,2*}, Lukas Engeler¹, Mauro Tonolla^{1,3,4}, Peter Lüthy⁵ and Nicola Patocchi⁶

Abstract

Background: In Ticino, a canton located south of the Alps in Switzerland, a surveillance programme on *Aedes albopictus* (*Stegomyia albopicta*) started in 2000 seeing that the region was considered at high risk of introduction based on the rapid spread of this mosquito in neighbouring Italy.

Methods: The surveillance programme, which is still ongoing, was adapted continuously to handle preventive measures of arrival, dispersal and establishment of this invasive species. The monitoring was based on ovitraps supported by reports from the population. The integrated control measures included removal of breeding sites, larvicide applications with *Bacillus thuringiensis israelensis* or diflubenzuron and, in some circumstances, adulticide applications with permethrin. These measures involved citizens, municipalities and Civil Protection Units.

Results: *Ae. albopictus* was first observed in 2003 in Ticino. We describe the strategies adopted and their adaptations to the evolving problem since year 2000. The approach used allowed keeping the mosquito densities at tolerable levels and below the threshold of autochthonous *Ae. albopictus* borne disease transmission. During the surveillance period, new typologies of breeding sites for *Ae. albopictus* have been discovered.

Conclusions: It was worth tackling the arrival of *Ae. albopictus* and adopting immediate control measures, followed by regular control measures after its establishment. Early intervention and prevention of the possible spread of the tiger mosquito over the territory avoided facing a crisis situation. This also reduced the difficulty of managing the situation and probably also reduced the overall cost if this had not been put in place.

Keywords: *Aedes albopictus*, Surveillance, Control measures, MALDI-TOF MS, Breeding site

Background

Towards the end of last century the Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) (Diptera: Culicidae), also known as *Stegomyia albopicta* sensu Reinert [1], was considered an invasive mosquito due to its capacity of taking advantage of global transportation of goods and traffic. This mosquito species 'annoys' humans because it can establish in urban settlements, bite at daytime often causing painful weals [2], and represents a sanitary risk due to its vector competence for various arboviruses and *Dirofilaria* sp. [3-5]. From its original distribution area in Southeast Asia, this

species managed to spread passively worldwide throughout Africa, the Americas and Oceania mostly transported as immature stages in artificial containers, especially used tyres [6-8].

In Europe the tiger mosquito was reported for the first time in Albania in 1979 [9] and later in Italy where it spread rapidly over the country and became permanently established [10,11]. The initial detection was reported from the city of Genoa in 1990 [12] and the first evidence of its establishment was observed in the Veneto Region in 1991 [13]. *Ae. albopictus* spread in Italy was mainly promoted by the trade of used tyres containing eggs [14] and road traffic which offered passive transport over long distances for adults [15]. Within ten years, the used tyre trade, road traffic and climatic conditions facilitated the rapid spread to most regions in North and

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Central Italy [2]. At that time, just a few surveys started with consequent control measures on *Ae. albopictus*, i.e. in some areas of the Veneto Region [16,17], around deposits of used tyres in the Regions of Emilia-Romagna and Piedmont [18], as well as in the touristic municipality of Desenzano del Garda, in the Province of Brescia [19]. Elsewhere in Europe the tiger mosquito was detected locally in Northern France in used tyre stock, probably imported from the USA or Japan [20]. Control measures were undertaken and elimination was achieved.

In 2000, anticipating the introduction of the tiger mosquito from Italy, the mosquito working-group (Gruppo cantonale di Lavoro Zanzare, GLZ) of the Canton Ticino (located South of the Alps in Switzerland), which was in charge of controlling floodwater mosquitoes in the Plaine of Magadino since its founding in 1988 [21,22], started a surveillance program. There was no evidence yet of infestation by *Ae. albopictus* in the Italian border regions with Switzerland nor of cross-border trade of used tyres. The main risk consisted of the passive introduction of adults by road traffic since the Canton Ticino is on one of the major traffic axes connecting South and North Europe. In addition, climatic conditions in the Canton Ticino are similar to those prevailing in Northern Italy, where the tiger mosquito was at that time established [2].

The objectives of the GLZ in the surveillance strategy on *Ae. albopictus* were to keep the density of the tiger mosquito at a bearable level for residents and tourists and to prevent autochthonous transmission of *Ae. albopictus*-borne diseases as well as uncontrolled use of insecticides. In this paper, we focus on the adopted surveillance strategy and the procedure used to challenge the invasion of *Ae. albopictus* from 2000, before the arrival of the mosquito in Ticino, until 2013.

Methods

The study site

The Canton Ticino has a surface of 2,812 km² and borders Italy to the West, South and East along 208 km. Its population is about 340,000 inhabitants and up to 60,000 cross-border workers travel daily from Italy. The Canton is divided into eight districts, five of them are included in the surveillance system: from North to South (see Figures 1, 2 and 3) 1) Riviera, (main city: Biasca); 2) Locarnese (main city: Locarno), the touristic region around the Swiss part of Lake Maggiore bordering with Italian provinces of Piedmont and Lombardy; 3) Bellinzonese (main city: Bellinzona); 4) Luganese (main city: Lugano) bordering with Italian province of Lombardy, and 5) Mendrisiotto bordering with Italian province of Lombardy (main cities: Chiasso and Mendrisio). Half of the population of the Canton lives in the Luganese district, followed by

Locarnese, Mendrisiotto, Bellinzonese and Riviera (<http://www3.ti.ch/DFE/DR/USTAT/index.php?fuseaction=definizioni.comuni-distretti>).

The climatic conditions where the tiger mosquito was established in northern Italy and in Ticino are similar. The mean summer temperature below 400 m.a.s.l. ranges between 20°C and 22°C with an annual mean temperature of 12°C, annual precipitations in Canton Ticino exceed with 1550–1900 mm the ones in Veneto (around 600–1000 mm), whereas for the summer months the precipitations are similar, 150–200 mm compared to 100–200 mm [23,24].

Surveillance system

The *Aedes albopictus* program, which has been fine-tuned and adjusted over the years, is based on two principal procedures: A. the survey on *Ae. albopictus*, B. the control measures that included treatments and the communication flow with the population of the Canton Ticino.

Methodologies applied during the surveillance system were based on the results obtained during surveillance activities (see results). Strategies adopted derived from what was known on the situation of the tiger mosquito at the moment, characteristics of the territory, economic resources and political interest.

The presence of *Ae. albopictus* can be divided into three successive periods. 1st period: this species was not yet detected, 2nd period: the mosquito appeared at several sites and had started to become established, 3rd period: the tiger mosquito had colonized wide urban areas.

Some strategies remained constant during all the periods, whereas others changed or were newly applied according to the period. The adopted strategies according to periods are presented in Table 1.

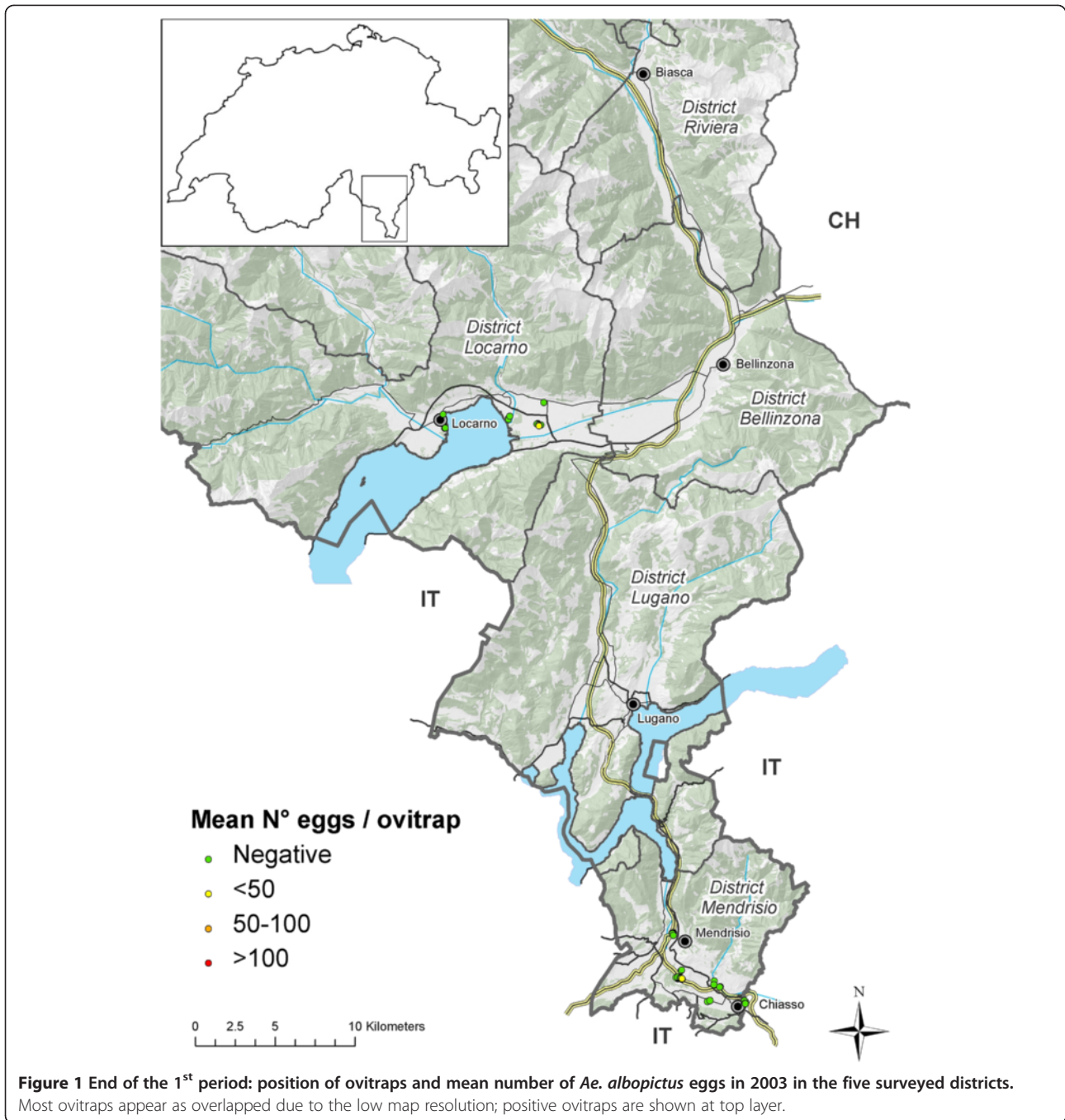
Data were organized in a relational database created with Microsoft Access, the georeference of the sampling site positions was taken from <https://map.geo.admin.ch> and maps were created with ESRI ArcGIS®.

A. Survey

The survey scheme is based on the positioning of traps in carefully defined sites, analysis of detected breeding places and evaluation of reports by residents.

Trapping

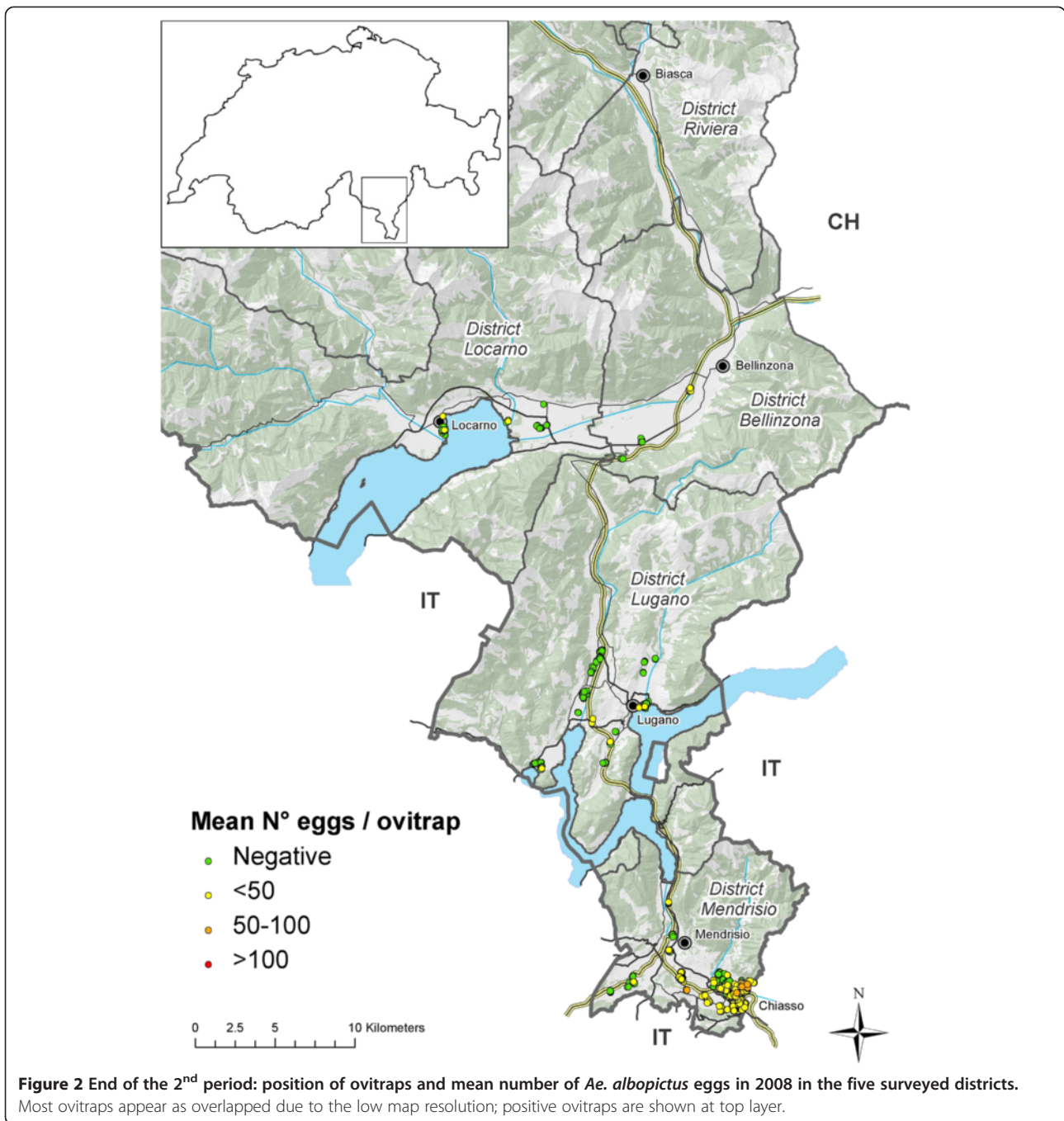
The principal tool used for detecting the presence of *Ae. albopictus* were the oviposition traps (ovitrap) where the mosquito can lay its eggs. Ovitrap consisting of black plastic containers of 1,5 l (Ramona Ø13/H12, Luwasa® Interhydro AG, Allmendingen, Switzerland) with an efflux hole on the top border were used. A slat of steamed beech wood (200 × 25 × 5 mm) was placed as an oviposition support in an angular position in the plastic container, which was filled with tap water. Ten



granules of VectoBac G[®] (Valent BioSciences, Libertyville, USA) were added in order to block the development of hatched larvae. The ovitraps were labelled with all the necessary information including phone number and the pledge not to remove it. Ovitraps were controlled by regular checking rounds. They were rinsed and filled with fresh water and granules. The wooden board was retrieved, labelled, wrapped into a plastic film and replaced. The boards were taken to the laboratory for examination for the presence of eggs. During the 1st period, checking rounds

occurred monthly except along the highway where they were checked bi-weekly. Similarly during the 2nd period, in control areas where the mosquito was not yet detected checking rounds occurred monthly. Otherwise the traps were controlled bi-weekly.

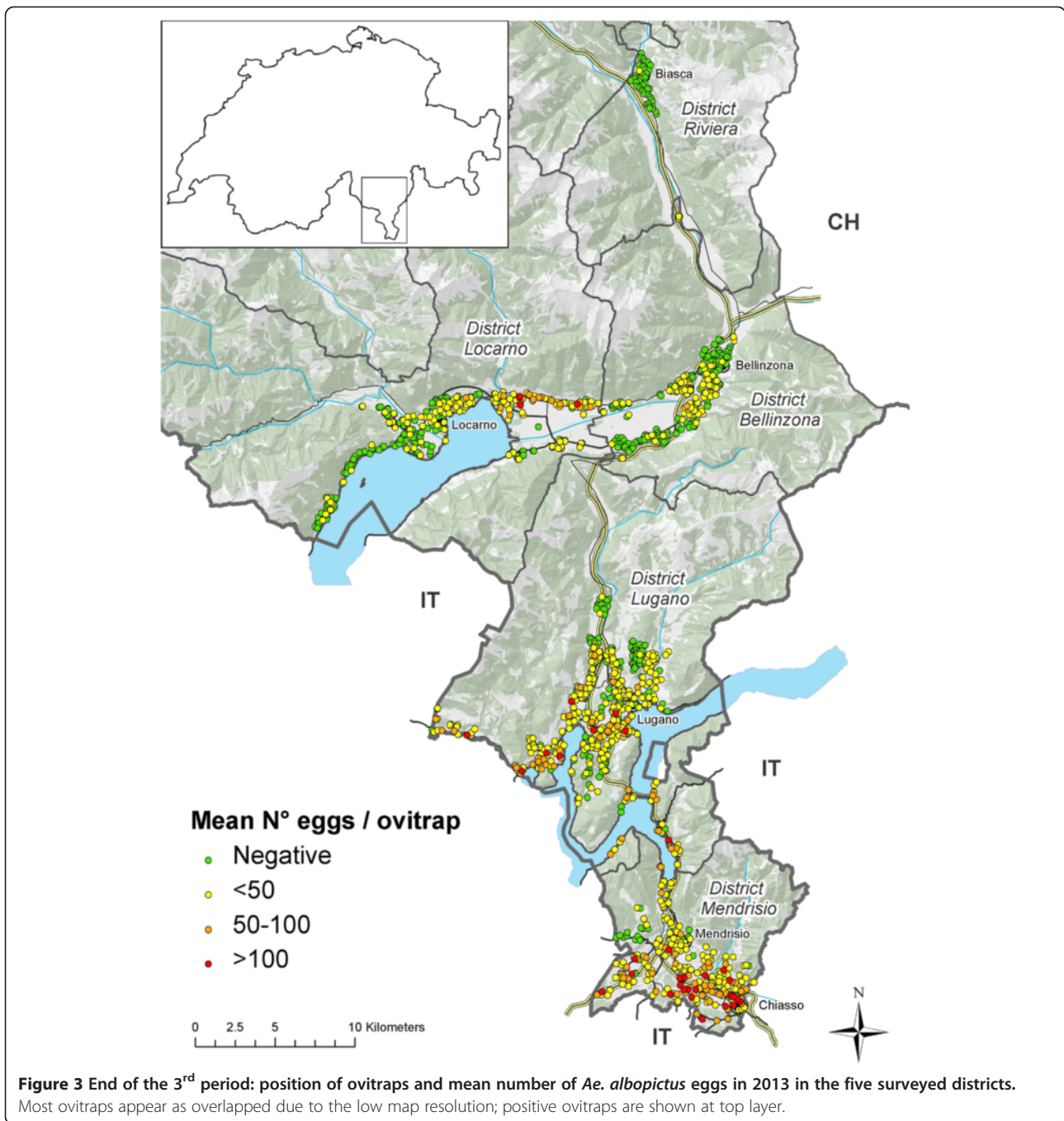
For adult sampling BG-sentinel traps (Biogents[®] AG, Regensburg, Germany) equipped with BG-Lure (Biogents[®] AG) and dry ice as attractants were placed on the ground close to vegetation or buildings for 24 h. BG-sentinel traps were used occasionally for comparison with ovitraps and



by biologists to estimate nuisance or to collect adults for detection of arboviruses [25]. For instant catches of adults an insect net or aspirator (Flashlight Aspirator, 2809C, www.bioquip.com) was used. To estimate nuisance, we performed human landing collections by counting the number of females landing within 15 minutes [26]. Captured adults were killed by exposure to dry ice then stored at -80°C for further virus analysis [25] or dry stored for conservation purposes.

Identification of mosquito species

Mosquito adults and larvae were identified using morphological keys [27,28], while eggs were morphologically identified under a dissecting microscope according to Zamburlini & Frilli [29]. Morphological identification of eggs was regularly confirmed by larvae hatched from the eggs or by using the Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry technique (MALDI-TOF MS) [30].



Selection of the control locations

The selection of the areas to survey for the presence of *Ae. albopictus* was based on two key points: the potential for the site to be an entry point for the mosquitoes by road traffic and the suitability of the site for the development of *Ae. albopictus*. Ovitrap were positioned close to or under vegetation or near buildings. An important factor was to select the most suitable places to position the ovitraps: they had to catch the mosquito at its arrival place, i.e. escaping from a vehicle, before

finding several other breeding sites and/or they had to be placed where adults could easily live, i.e. presence of hosts for blood meal, shadow, etc. We also took into consideration the priority of a location to be checked taking into account the nuisance and the impact of mosquitoes on sanitary and economic situations, i.e. schools, public parks, touristic places.

During the 1st period there was still no record on the presence of *Ae. albopictus* in the territory. The territory was first screened with a general look on major breeding

Table 1 General schema of the *Aedes albopictus* surveillance strategies and procedures adopted in Canton Ticino (Switzerland) from 2000 to 2013

Surveillance system	First period (2000-2003)	Second period (2004-2008)	Third period (2009-2013)	
Survey	survey aims	detect mosquito presence at it's arrival	intercept mosquito at arrival and analyse it's spread	analyse mosquito's spread and oversee it's densities
	surveyed areas	points of entry and development (major parking places, cemeteries, parks, etc) and resting places along the highway	points of entry and development (major parking places, cemeteries, parks, etc), industrial areas, whole municipalities (if positive), resting places and parking's along the highway	whole municipalities with grid system, resting places and parking's along the highway
	site selection for trap positioning	GLZ	GLZ	GLZ and municipalities
	traps managing	GLZ	GLZ	municipalities
	collection with other traps	no other traps used	BG-sentinel, Human Landing Collection	BG-sentinel, Human Landing Collection
	contact with residents	phone, mail and e-mail (shipping suspicious insects)	phone, mail and e-mail (shipping suspicious insects)	phone, mail and e-mail (shipping suspicious insects) and web page
	management of resident breeding sites inspection	GLZ	GLZ	GLZ and municipalities
	warm season survey period	mid-June till mid-October	April till mid-October	May till end-September
	cold season survey period	no survey	no survey	December till May (GLZ)
	Control	control aims	elimination	elimination or control of densities
decision, responsibility and supervision of treatments		GLZ	GLZ	GLZ
treatments timing		once positive	once positive	once positive or monthly if most of municipality was found positive
treatments areas		around positive site	around positive site or the entire municipality (private and public areas)	the entire municipality (private and public areas)
treatments execution		GLZ	GLZ (with help of municipality and Civil Protection)	municipality with help of Civil Protection under the supervision of GLZ
larvicide used		<i>Bti</i>	<i>Bti</i> or diflubenzuron (for large scale treatments)	diflubenzuron or <i>Bti</i> (if requested from the municipality)
adulticide used		permethrin	permethrin	permethrin (only in schools etc. or for epidemic risk reduction)
removal of breeding sites		GLZ	GLZ and municipality	GLZ and municipality (ordinance)
areas concerned for removal of breeding sites		around positive site	door-to-door	when reported
information		media (newspapers, radio and television)	media (newspapers, radio and television), web page and specific leaflets	media (newspapers, radio, television), web page, specific leaflets and instruction on <i>Bti</i> tablets usage for citizens

GLZ: Gruppo cantonale di Lavoro Zanzare, see text.

Bti: *Bacillus thuringiensis israelensis*.

sites favourable for settlement and entry points for the mosquito, i.e.: highway resting places, parking places of the main supermarkets, deposits of used tires, main parking sites, airports, camping sites, customs, train stations, ports, recreation sites. At the same time we

checked the most representative places where this species could easily develop, i.e. cemeteries, greenhouses, deposits for used tires and urban parks were inspected.

When the mosquito appeared in the territory (2nd period) the survey was extended to other possible entry

points, that is to say industrial areas, considered significant for the risk of introduction due to the daily traffic of goods and workers (in the case of canton Ticino, mainly cross-border workers) from Italy. In addition the survey was extended to surrounding areas where the mosquito was detected by traps, residents' reports (see *Reporting by residents*) or personal observation of survey technicians'.

During the 3rd period, when the tiger mosquito started to colonize urban areas, there was the need to have a finer net of ovitraps to closely monitor and map the dynamic of the spread of *Ae. albopictus*. The survey on the highways stops was maintained, but in addition the survey was extended to the entire urbanized areas of municipalities where the mosquito was already detected or of those municipalities considered at risk of introduction. In this case a grid system was adopted to standardize the distribution of ovitraps on the territory. The entire cantonal territory was divided into a grid of 250 × 250 m units, but only units concerning urbanized and industrial areas were considered. Each unit was designed as checkpoint where ovitraps were positioned. Since it was not possible to survey all municipalities of the Canton Ticino, there was the need of giving priority to municipalities and then selecting some of them according to the available budget. Adopted criteria and their priority scale are indicated in Table 2, whereas priorities appear in Table 3. The upper altitude limit was set at 400 m a.s.l. because the higher densities of *Ae. albopictus* in Italy were observed below this limit [31], although the tiger mosquito was also reported as high as 600 m a.s.l. Every year municipalities or new territories of a municipality (>400 m a.s.l.) were added following the same priority criteria.

Number of ovitraps in the control location

The number of ovitraps in a location, i.e. parking place, etc., depended on the characteristics of the site (e.g. different areas for car stopping, risk of manumission). Therefore several traps (up to 15) were placed in the same thematic area in order to cover all the arrival and spreading points in the 1st and 2nd periods. Surveillance of locations colonized by the mosquito was intensified and extended to surrounding areas once *Ae. albopictus* was discovered at the end of the 1st period and during the 2nd period.

With the grid system, during the 3rd period, accommodating two traps every checkpoint was considered as a minimum to detect the presence of *Ae. albopictus*, based on observations made during the 2nd period. Once the tiger mosquito became established in a municipality (>80% positive checkpoints) the number of ovitraps was reduced to a single one per checkpoint in order to reduce the amount of work, but maintaining the accuracy of data. The choice of the maintained ovitrap was done

randomly. Hence, municipalities newly colonized by *Ae. albopictus* maintained 2 ovitraps/checkpoint, whereas municipalities with established tiger mosquito populations had one ovitrap/checkpoint. At the end of 3rd period, due to budget restrictions, amount of work and wide spread of the tiger mosquito only one trap/checkpoint was positioned.

Period of trapping

During all periods, activities were concentrated during the warmest months that varied depending on the period (Table 1), when the probability to detect the presence of *Ae. albopictus* was the highest, according to results of Italian surveys [2] and to results obtained during the survey in Canton Ticino.

During the last two years of the 3rd period, additional winter surveys were set up with 16 ovitraps positioned from December till April, controlled monthly, in the most positive sites, chosen in the most representative municipalities of two different districts.

Checking rounds controlled

For 1st and 2nd periods all the paddles of the ovitraps were controlled in the laboratory, while at the end of 3rd period (2013) the analysis for the presence of eggs was done only every second checking round and the regular biweekly analysis was maintained only for one municipality/district, i.e. about every 100 km². The municipality was chosen among the ones, which provided regular data and were located centrally in the district. The remaining labels were stocked for contingent further analysis, like doubts for treatment areas, risk of epidemic or studies on a specific area.

Identification of breeding sites

During fieldwork with ovitraps it was important to gain an overview of the potential breeding sites. Therefore places where water could accumulate had to be checked, focusing especially on those containing < 200 l [4], i.e. catch basins, plant saucers, drums, buckets, tarpaulins, tyres, bathtubs, but without neglecting the larger ones. Tools used to check these places were standard pint dippers (model 1132, BioQuip Products, Rancho Dominguez, USA) or a Pasteur pipette with the tip cut off or a fish net for aquariums. Larvae were stored in 70% ethanol whereas pupae were kept in the laboratory in plastic trays at 28°C, with 12 hours/daylight, until they emerged as adults and were identified to species.

Reporting by residents

The system of trapping was insufficient to cover the whole territory. Therefore community participation was organized: residents were asked to signal the occurrence of *Ae. albopictus*. Experts of the Mosquito Working

Table 2 Criteria and scores adopted for the choice of municipalities that were to included in the surveillance system

positivity of ovttraps¹⁾	negative	0
	close to municipality with ovttraps positive once a year	1
	close to municipality with ovttraps positive several times a year	2
	close to municipality with ovttraps regularly positive	3
	municipality with ovttraps positive once a year	4
	municipality with ovttraps positive several times a year	5
	municipality with ovttraps regularly positive	6
	stops on the highway	hard shoulders
	gas stations	2
traffic of wares		
commercial customs ²⁾	≤ 0.1 millions/t/year of wares	3
	0.1 < x ≤ 2 millions/t/year of wares	6
	2 < x millions/t/year of wares	9
carriage free ²⁾	x = number of points	3x
companies with customs control ²⁾	1 company	2
	2 < x ≤ 4	4
	5 ≤ x	6
transport companies	x = number of companies	x
cargo vessels ³⁾	x = number of SBB-Cargo (train cargo service)	x
<i>Total value = commercial customs value + carriage free value + companies with customs control value + transport companies value + cargo vessels value</i>		
attraction points		
sport centres ⁴⁾	x ≤ 5	1
	5 < x ≤ 10	2
	10 < x ≤ 20	3
	x > 20	4
shopping centres	x = number of shopping centers	2x
tourist attractions ⁵⁾	x = number of tourist attractions	x
train stations ⁶⁾	regional	1
	international	2
airports ⁷⁾	x = number of airports	x
<i>Total value = sport centres value + shopping centres value + tourist attractions value + train stations value + airports value</i>		
cross-border workers⁸⁾	x = number of workers/urban settlement area (km ²)	x
population⁸⁾	x = number of inhabitants/urban settlement area (km ²)	x
entering cars⁸⁾	≤ 1 million persons/year	1
	1 < x ≤ 5 millions persons/year	2

Table 2 Criteria and scores adopted for the choice of municipalities that were to included in the surveillance system (Continued)

	5 < x ≤ 10 millions persons/year	3
	10 < x ≤ 60 millions persons/year	4
traffic on highway exits	≤ 20'000 cars/day	1
	20'000 < x ≤ 50'000 cars/day	2
	> 50'000 cars/day	3
<i>Total value = customs crossing value + traffic on highway exits value</i>		
overnight stays⁸⁾	x = number of overnight stays/urban settlement area (km ²)	x

- ¹⁾survey's data (previous years).
- ²⁾AFD - Amministrazione federale delle dogane (Swiss custom administration).
- ³⁾SBB-CFF-FFS.
- ⁴⁾GIS - Geographical Information System.
- ⁵⁾Ticino turismo (Ticino tourism).
- ⁶⁾SBB-CFF-FFS.
- ⁷⁾Swiss Topo.
- ⁸⁾USTAT - Ufficio di statistica del Cantone Ticino (Statistic office Canton Ticino).

Group (GLZ; see *Actors of the surveillance system*) were at disposal to answer questions and to determine insects collected by residents. Citizens were asked to send specimens to the laboratory. When the specimen was identified as *Ae. albopictus*, GLZ experts went to the field for further analysis. If a municipality outside the survey resulted positive, it was included in the survey in the following season.

B. Control measures

Control measures consist of integrated strategies to eliminate or reduce the presence of *Ae. albopictus* including information to authorities and residents, breeding site removal and treatments.

Table 3 Priorities given to municipalities in joining the surveillance system

Index	High priority	Medium priority	Low priority
positivity	>3	3	<3
stops on the highway	>1	1	0
traffic of wares	>9	4-9	<3
attraction points	>9	7-9	<6
cross-border workers	>800	500-800	<500
population	>3500	2500-3500	<2500
entering cars	>4	3-4	<3
overnight stays	>200000	100000-200000	<100000

Note: If less of 5% of municipality territory is below 400 m a.s.l. the municipality was not included in the survey. For the other municipalities a decreasing gradient of priority index going from positivity index to overnight stays index was applied.

Information

Urban areas in Canton Ticino are composed mostly of small private properties therefore to prevent the establishment of the tiger mosquito a targeted information campaign for the population was necessary. Mass media like TV, radio and print media were used from the beginning of the survey to inform the population on the characteristics and risks related to *Ae. albopictus* during the summer season. From the 2nd period, information on the situation about the mosquito and the measures undertaken by authorities was added, and citizens were asked to avoid having domestic breeding sites. This information belonged to an annual scheduled communication flow, which started after a press communication released by the GLZ around May and was repeated in July and beginning of September in order to reach as many people as possible. In addition, a GLZ web page (www.ti.ch/zanzare) was built containing downloadable general information on *Ae. albopictus*, annual reports of GLZ, FAQ format and leaflets targeting citizens. The leaflet, available in Italian, French and German, was a key tool in the fight against *Ae. albopictus*. They contained explanations on the risks related to the tiger mosquito (nuisance and vector capacity), the potential domestic breeding sites and the measures to undertake to prevent the reproduction of the mosquito (avoid, remove or treat breeding places). In addition from the 3rd period, residents were asked to treat breeding sites for *Ae. albopictus* they could not remove, therefore a list of *Bacillus thuringiensis israelensis* (*Bti*) tablets available on the market and their suggested dilution were included on the web page. In all documents phone numbers for notifications of the mosquito presence or request of information were available. Furthermore in the 3rd period, municipality authorities transmitted annual information to residents in May, regarding the situation of *Ae. albopictus* presence in their territory, the measures to be taken, the dates of planned treatments, suggestions. The same information had to be present on the municipality webpage as well.

Removal of breeding sites

Removal of breeding sites was considered as a first priority of control measures. Residents were urged to remove them with a specific door-to-door distributed leaflet, also available on the GLZ web page. During the 2nd and 3rd periods (see results), in areas concerned by door-to-door treatment actions, breeding sites were removed directly by GLZ, Civil Defence Units or municipality workers after agreement with the residents. During the 3rd period the GLZ suggested the municipalities to produce a specific ordinance not permitting uncared breeding sites for the tiger mosquito on the municipality territory. Therefore municipality workers were

able to verify the presence of breeding sites in private domains and possibly to give penalties.

Treatments

Larvicides and adulticides were applied to control *Ae. albopictus*. The focus was clearly on larvicides because the immature stages were present in well-defined habitats. Furthermore the effect of larvicides was longer lasting and impacts on the environment were reduced. Larvicide applications were executed always when *Ae. albopictus* was detected. Adulticides were only carried out in parallel to larvicides when the mosquito was not yet established (end of 1st and 2nd periods) or when it was necessary to protect sensitive places like schools, kindergartens and senior residences (3rd period).

It was decided to use two active ingredients for larvicide treatments: *Bti* or diflubenzuron and for adulticides pyrethroids like permethrin or cypermethrin. The applied products were the only ones whose use was permitted against *Ae. albopictus* in Switzerland. In fact, not all the products used are registered for mosquito control, but the GLZ received a temporary permission to use some biocides for the control of the tiger mosquito. For *Bti*, 30 granules/catch basin (average water content of 50 l water) of VectoBac G[®] (Valent BioSciences) were used. A concentration of 0.025 ml/catch basin was applied when the liquid formulation (Solbac[®], Andermatt Biogarten AG) was applied, and 1 tablet per 20 catch basins was used for the solid formulations (Solbac-Tabs[®], Andermatt Biogarten AG; Bio Garden Trauermücken-Stopp[®], Migros-Genossenschafts-Bund; Coop Oecoplan Biocontrol Mücken-Tabletten[®], COOP). When diflubenzuron (Device[®] SC-15, Paradiffusion SA) was used, 10 ml of a 2 ml/l solution were applied either with a dosage bottle (Schnelldosierflasche cat. No. 3807, Semadeni Plastics Market AG, Ostermundigen, Switzerland) or with knapsack sprayer in each catch basin (50 l). The quantity of applied insecticide was reduced proportionally if the capacity of the breeding site was smaller. Diflubenzuron was applied only if no rainfalls were forecasted for the next 3 days. During the larvicide applications all permanent breeding places, such as catch basins, were treated, whereas not permanent ones were, if possible, removed.

VectoBac G[®] was the only *Bti* larvicide applied in not extensive treatments during the end of the 1st period and the 2nd period, whereas during treatments on large surfaces at the end of the 2nd period and during the 3rd period a liquid formulation was preferred and a longer lasting insecticide was necessary in order to avoid frequent applications. The IGR diflubenzuron was introduced, which permitted application lasting for about one month [32,33]. *Bti* liquid formulation (Solbac[®]) was used only in case of unfavourable weather forecast to replace

diflubenzuron in already planned larvicide applications or in case a municipality expressed the wish not to apply IGR. In this last case, application on catch basin had to be repeated every week, because the effect of used *Bti* formulation is immediate and has no persistence. The insecticide application had to interrupt the mosquito aquatic phase that, during summer climatic condition lasts about a week. *Bti* tablets were used by residents to treat their domestic breeding sites, with the recommendation to apply this insecticide once a week, from May till the end of September.

Adulticide applications were executed during the evening hours on the vegetation surrounding positive locations up to 2 m above the ground level with a backpack sprayer using permethrin (Matil®, Maag AG) or cypermethrin (Cypermethrin®, Sintagro AG), following dilutions recommended by the manufactures. Applications have to hit the target with limited side effects on non-target organisms, therefore: in order not to affect their waters, adulticide treatments were not performed close to rivers or lakes, in addition grasslands were barbed before, so they did not need to be treated and edible vegetation was not concerned to avoid any health problem to the population.

The adulticide and larvicide applications were performed on a radius of 100 m and 200 m around positive traps respectively, shortly after the detection of *Ae. albopictus*, i.e. in the 2–4 weeks following detection in order to eradicate the mosquitoes when the tiger mosquito was not yet widely established (1st and 2nd periods). Large surfaces applications using both larvicide and adulticide applications were performed only at the end of the 2nd period. In this case, all treatments were performed door-to-door and the population was informed through media (television, radio and newspapers) as well as through a leaflet in their letterbox. Treatments occurred twice in the warm season: around the beginning of June and between the end of August and the beginning of September. During the 3rd period, when *Ae. albopictus* was already widespread, the treatment goal was no longer the mosquito elimination but rather the control of its density. Treatments were planned already during wintertime. Three main seasonal interventions were planned on the territory in accordance with the availability of the regional Civil Protection members (see *Actors of the surveillance system*): a) between mid-May and mid-June, b) in July (not always possible) and c) between mid-August and mid-September. The first treatments aimed at targeting the first juvenile stages, the second to control densities before the summer peak of August–September, while the third aimed to reduce the adult population laying overwintering eggs. The surface to be treated for the first intervention was based on data from the survey of September of the previous year, while the treated surfaces for the second and third interventions fitted on data from the on-going survey. Until

2013 (end of the 3rd period), interventions were lead in public and private domains, i.e. treating catch basins on public streets and treating or removing door-to-door every breeding site, while since 2013 applications concentrated only on public areas. Municipalities infested by *Ae. albopictus* were asked to apply larvicides on a monthly (diflubenzuron) or weekly (*Bti*) basis in public areas from May till October. Concomitantly citizens were asked (through specific information leaflets) to treat their private property with *Bti* tablets.

Actors of the surveillance system

The pre-existing Mosquito Working Group (GLZ) took the responsibility to start the surveillance system on *Ae. albopictus* among its general duties on mosquitoes. By the end of the 2nd period, the GLZ was officially in charge of the surveillance on *Ae. albopictus* and a specific group was added to face problematic connected with the presence of the tiger mosquito. This group included cantonal representatives of health, tourism, territory, environment, veterinary institutions and concerned municipalities.

All the morphological determination were lead by specialists of the GLZ for all the three periods, whereas survey and control measures in the 3rd period by the increasing of the presence of the tiger mosquito in the territory were actuated by different actors: GLZ, municipality workers and Civil Protection Units.

For the 1st and 2nd periods all the survey was lead by a biologist of the GLZ employed 50% for the 1st period and 100% for the 2nd. Treatments were planned and carried out by the biologist with the help of 2 workers of the GLZ. During the last year of the 2nd period treatments on large surfaces in concerned municipalities were started. In this case workers and soldiers of the Civil Protection were coordinated by the biologist of the GLZ, with the help of municipality. All kind of treatments was performed under the supervision and organisation of GLZ workers patented for use of biocides.

For the 3rd period two work units (80% each) were employed during the year and 4 other work units (50% each) for the six warm months were added.

During the 3rd period the surveillance work on *Ae. albopictus* obtained the collaboration of the concerned municipalities. According to the health care law in the Canton of Ticino, the municipalities have to take measures to fight against organisms that transmit diseases on their territory, and GLZ acted as consultant and coordinator of the activities. Therefore a constant communication flow was established between municipality authorities and GLZ. Each year, in January, an annual report and a report specific for each municipality were sent. In addition, between the end of March and mid of April, all municipality delegates were invited at annual

meetings with representatives of regional Civil Defences in order to discuss and adjust methodologies and strategies as well as the time schedule for control measures. During the surveillance season the GLZ was at disposal of the municipality authorities for any kind of problems and all communications from citizens concerning *Ae. albopictus*, which were supervised by the GLZ.

Technicians of the surveillance programme together with municipality delegates selected the places for ovi-traps. Personnel of each municipality carried out the routine check of ovi-traps while the counting of eggs on the collected wooden paddles was done by the GLZ personnel. Therefore in 2009 the GLZ requested the active participation in the survey and control measures of municipalities considered at major risk. The grid system applied on the territory and the planned treatments permitted to rationalize and delegate part of the work to municipality personnel, while the GLZ maintained the supervision of the whole process.

In all surveys, the highway was included as well, but surveyed and controlled by GLZ personnel only.

Civil Protection joined regularly the municipalities in the treatment actions, which were planned already during wintertime. Three main seasonal interventions were planned on the territory in accordance with the availability of the regional Civil Protection members.

Results

In this paper the results are described and divided into three successive periods according to the presence of *Ae. albopictus* in the territory (see *materials and methods*). The surveillance results obtained during these successive periods were the basis for decision for further measures, that is to say they were the basis for the different methodologies applied during different periods and described in the materials and methods section.

1st period (2000–2004)

Being aware of the high risk of passive introduction, we initiated a proactive monitoring of *Ae. albopictus* in 2000. At that time, there was no sign of the presence of *Ae. albopictus*, but there was a high risk of passive introduction through road traffic from Italy. Therefore surveillance was mainly focused on possible points of entry at the state border and along the South–North highway (E35), which is the only highway in the Canton. At the same time the most representative places where this species could easily develop, i.e. cemeteries, greenhouses, deposits for used tires and urban parks were inspected.

In year 2000, the territory was first screened with a general look on major breeding sites favourable for settlement and entry points from Italy. Since *Ae. albopictus* was not observed, some checking points were left aside the following years, because they did not appear as a priority.

In fact, deposits of used tires had no trade with foreign countries, cemeteries were mostly very dry and greenhouses did not represent a main place for traffic stop. The survey was then extended to other main traffic parking places.

During this start monitoring period used tires were also adopted as traps, but their managing was difficult compared to ovi-traps and this method was not further used.

In 2003, a total of 34 ovi-traps were positioned at the beginning of July until mid-September (Figure 1). Principally 6 bi-weekly checking rounds were conducted but some ovi-traps were controlled just monthly. Hence a total of 166 ovi-traps were analyzed for egg presence on their slats. Seven ovi-traps turned to be positive. In fact *Ae. albopictus* was found for the first time between mid-July and end-August 2003 in Switzerland in four ovi-traps, in the Mendrisiotto, at the first service station on the highway entering Switzerland from Italy (Figure 1). Similarly three ovi-traps were found positive between end-August and mid-September in a small airport close to Lake Maggiore, in the Locarnese district. These ovi-traps had a mean number of eggs <50. After these observations, the number of ovi-traps was doubled in order to improve the detection for the following checking rounds.

Larvicide and adulticide applications were performed after the discovery of *Ae. albopictus*, end-August on the highway and mid-September in the airport. No *Ae. albopictus* was found after the treatments till the end of the surveyed season, i.e. mid-October 2003.

In the following year traps were set on the northwards service station on the highway and at the Lugano-Agno airport, in the Luganese district. A total of 57 traps were set, resulting in 465 checks during the season, of which 23 resulted positive to *Ae. albopictus*. The presence of the mosquito was confirmed at the small airport of Magadino, in the Locarnese district and in the first service station on the highway coming from Italy in the Mendrisiotto district. Additionally the mosquito was found in the second service station on the highway and in a cargo train station in the Mendrisiotto district. Again control measures were undertaken.

2nd period (2005–2008)

Following *Ae. albopictus* discovery in Canton Ticino in 2003, and confirmation of its presence in 2004 on the highway and in one airport (Magadino), as well as its appearance in the cargo train station, it was decided to implement the survey on other possible entry points. Hence the survey was extended to the industrial areas, where most of the cross-border workers coming from Italy stop, and to houses surrounding the service stations along the highway, when they were found positive.

During the 2nd period, *Ae. albopictus* was detected sporadically at several locations and started to settle in the territory (Figure 2). Therefore, the survey was extended to

additional industrial areas, to the urban areas of positive municipalities and in all resting places along the highway.

In 2007 the town of Chiasso, at the border with Italy was found positive thanks to a report from a citizen by the end of September 2006. In 2008, two additional municipalities of Mendrisiotto district close to that border area, Morbio Inferiore and Vacallo, were included in the surveillance system. In 2008, tiger mosquitoes flying directly across the border from the Italian municipality of Maslianico (Province of Como) to Vacallo were observed and most of the inspected catch basins in the Italian municipality (Maslianico) were found positive to *Ae. albopictus* (Dr Romeo Bellini and Eleonora Flacio, personal observations).

At the end of the 2nd period, in 2008, 466 ovitraps were positioned from the beginning of April to mid-October (Figure 2). A total of 4,919 controls of ovitraps were carried out in 14 bi-weekly checking rounds and 436 turned to be positive. *Ae. albopictus* was prevalently detected in Mendrisiotto, but it appeared as well in the Luganese and Locarnese districts, and the ovitraps at the resting places along the highway started to be constantly positive to the mosquito. The average number of eggs/season varied but did not reach more than 100, the highest being in the southern part of the studied area. The first service station on the highway in Mendrisiotto was still positive, whereas the airport close to Lake Maggiore not.

Until 2007 larvicide applications were performed by GLZ workers with VectoBac G[®] and continued on highway resting places until the end of the 2008. In July 2008, we started applications on large surfaces with the help of municipality workers and soldiers of the Civil Protection therefore a longer lasting insecticide was necessary in order to avoid frequent applications, thus the IGR diflubenzuron was introduced. If the positive areas were restricted, treatments managed generally to eliminate the mosquito. One such example is the main park in Lugano, Parco Ciani, where the tiger mosquito was found for the first time in July 2005, disappeared after the treatment and was found again only at the end of June 2008. Similarly to the 1st period, treatments were carried out shortly after the detection of *Ae. albopictus*, i.e. in the 2–4 weeks following mosquito detection in order to eradicate the mosquito or control its density.

3rd period (2009-up to now)

During the 3rd period, when the tiger mosquito started to spread in the territory, municipality authorities were directly involved in the survey from the beginning of the colonization of the tiger mosquito. The grid system to standardize the distribution of ovitraps on the territory was adopted in the municipalities involved in the surveillance program. The active requested participation of the municipalities for the survey as well as for the control measures allowed from the beginning to radically increase

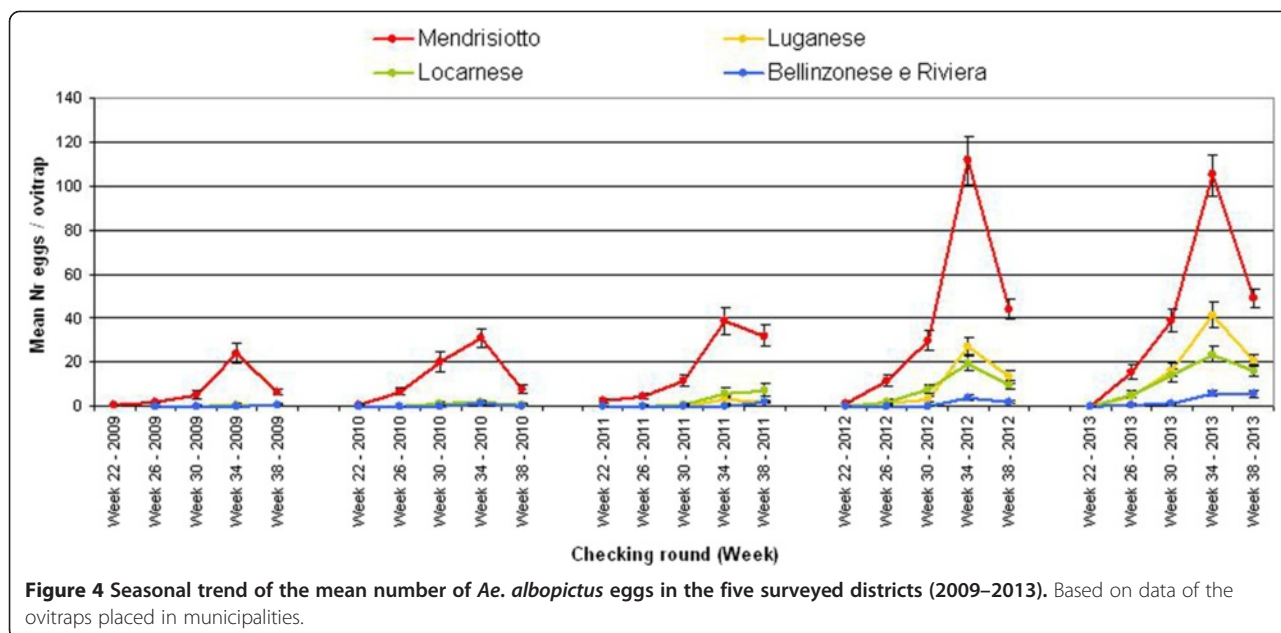
the surveyed area and gave a better information accuracy regarding the presence of the tiger mosquito. The number of traps set was raised from 466 in 2008 to 1,241 in 2009, the number of controls on ovitraps from 4,919 to 10,526 and the number of involved municipalities increased from 21 in 2008, (when the entire urban settlement was not yet considered and no grid system was in place) to 37 in 2009 when the entire urban settlement with the grid system was arranged. In 2013 the number of involved municipalities was 61, covering 76.5% of the population of the Canton Ticino.

During the last two years of the 3rd period, additional winter surveys were set up with 16 ovitraps positioned from December till April, controlled monthly, in the most positive sites, Chiasso and Lugano belonging to Mendrisiotto and Luganese, respectively (Figure 3). However, the winter surveys did not show any activity of *Ae. albopictus* in the ovitraps. Active *Ae. albopictus* adults were found from mid-May to beginning of June until mid-November.

Ae. albopictus started to be found in large urban areas along the southern border with Italy and more frequently in cities like Lugano and Locarno (Figure 3).

The screening of the ovitrap paddles for mosquito eggs by the GLZ became more and more time-consuming (up to 13,418 paddles analyzed in 2010), therefore a reduction of the number of ovitraps was applied for municipalities with >80% of positive ovitraps over the season. In addition a reduction of checking rounds analyzed for eggs was applied in 2013 (see *methods*). During the 2013 survey (Figure 3), 1,389 ovitraps were positioned in the field. A total of 7,069 controls of ovitraps were carried out among the checking rounds analyzed during the season and 2,081 turned to be positive. Positive ovitraps appeared all along the highway. The average number of eggs/season increased and average densities of more than 100 eggs per ovitrap during the season were observed at various locations, whereas in most surveyed locations densities used to be lower. The mosquito was widespread in most regions, except in the Riviera district where only one or two traps were positive to *Ae. albopictus* during the survey season. In the Mendrisiotto district, where the mosquito appeared first in 2003 (Figure 2), almost all ovitraps were positive, but high egg densities were present mainly around the service stations on the highway and along the border with Italy. The airport close to Lake Maggiore remained negative (Figure 3).

Figure 4 shows the seasonal trend of the mean number for *Ae. albopictus* eggs subdivided into the five districts of the municipality survey. A maximum of around 110 eggs/ovitrap was observed during the peak of activity. The district most concerned by *Ae. albopictus* during the 3rd period was Mendrisiotto, the southern region of Ticino. In 2012 and 2013 the Luganese and Locarnese



districts reached egg densities, which were previously observed in Mendrisiotto only. In Bellinzonese and Riviera, the mosquito densities increased but remained relatively low. Between 2011 and 2012 there was a prominent increase in egg densities, which remained constant during summer 2013.

Citizen reports on the presence of *Ae. albopictus* outside the surveyed area

The survey was based on results of ovi-traps as well as reports of citizens who called or sent emails to the GLZ to communicate the suspected presence of *Ae. albopictus*. The GLZ received around 500 reports/year. The reports usually came from areas where the presence of the tiger mosquito was already known, but every year 1 or 2 reports allowed confirmation of the presence of *Ae. albopictus* outside the survey system. Residents contacted the GLZ not only for suspected cases, but also for any counselling regarding the tiger mosquito, i.e. treatments within their property, nuisance, risk of diseases, etc. About 35% of the contacts where phone calls, 35% were emails, while 10% were made directly to the operators of GLZ during their field work and about 20% were letters containing insects arriving by post. Feedback from GLZ were given within two weeks. Usually reports arrived from municipalities having lower priority, which had not been included in the survey because of limited financial resources.

New breeding sites

Inspected breeding sites corresponded mostly to what was known from the literature [4] and other survey systems, i.e. containers with < 200 l of water, but also bigger breeding sites such as oil sorters close to the highway

and in large parking places could be detected. These places contained thousands of litres of water and included several connected underground tanks, close to the surface, with small openings in the manhole covers for inspection. All the other inspected oil sorters not closed at the surface were negative for *Ae. albopictus*. Larvae of the tiger mosquitoes were also found in flooded basements of building yards and in other receptacles containing thousands of litres of water, such as tanks, broken fountains, cisterns. All these large water containers were situated in shadow under vegetation.

Adults were detected in wooden areas, surrounding colonized villages, but no breeding sites could be detected: neither tree holes containing water nor general waste that could act as breeding sites [34].

Nuisance

In response to citizen phone calls claiming a nuisance caused by *Ae. albopictus*, GLZ workers placed BG-sentinel traps around the affected houses. For example, a total of 821 females were caught between end-August and mid-September 2013 during 37 sampling sessions, corresponding to an average of 22 females/day. Nuisance prompted citizens to adopt control measures suggested by GLZ, i.e. most of them started to remove breeding sites after experiencing the nuisance due to tiger mosquitoes.

Egg identification

An extensive survey applying the hatching technique on all positive paddles of the ovi-traps is too time-consuming, therefore hatching was only used sporadically. In particular, hatching was performed during the 2nd period, for distinguishing morphologically, under a

dissecting microscope, eggs of *Ae. albopictus* from the ones of *Ae. geniculatus*, a species frequently laying its eggs on the wooden paddles of the ovitrap [29]. In 2013, eggs identified morphologically as *Ae. geniculatus* were confirmed using MALDI-TOF MS technique [30]. Among 325 samples morphologically identified as *Ae. geniculatus* only one was shown to belong to *Ae. albopictus*. In addition, for the eggs identified as *Ae. albopictus*, 3 to 5 positive traps, randomly chosen for each municipality were checked three times during the season. Samples came from 46 municipalities among 61. A total of 3,334 eggs were analyzed and all were confirmed as *Ae. albopictus* with the Mass Spectrometry technique.

Discussion

Considering the rapid spread of *Ae. albopictus* in Italy, the GLZ realized that the Canton Ticino became a region at high risk of invasion. Therefore, a preventive surveillance system was implemented in 2000 in order to face the problem from the beginning. Continuous introduction followed by a local diffusion prompted the GLZ to adopt a dynamic and flexible surveillance system, which can be resumed in three major periods of time corresponding to the level of the spread of the tiger mosquito. Those periods fit to the three scenarios described in the recent published guidelines of the European Centre for Disease Prevention and Control [35] and the strategies we adopted from 2000 to 2013 are similar to the ones indicated in the ECDC guidelines and in the ones of WHO/EMCA [35,36].

The surveillance system, which is still ongoing, allowed the first detection of *Ae. albopictus* in Canton Ticino in 2003 [37] and the management of its spread [38-40]. Ovitrap are the main tool for monitoring species belonging to the subgenus *Stegomyia*, i.e. *Ae. aegypti* (Linnaeus, 1862) and *Ae. albopictus* [26,41]. Ovitrap detect mosquitoes even at low densities, are not expensive and are easy to handle even by unskilled staff. To be used in a large-scale survey a compromise between attractiveness for the mosquito (colour, shape, odours, persistence of water), safeness, i.e. not being breeding sites themselves, simplicity in handling and availability on the market has to be found. In fact other trap methodologies were tried, such as the trap for adults BG-sentinel (Biogents AG, Regensburg, Germany) when the tiger mosquito started to be detected in urban areas in 2008. BG-sentinel traps equipped with BG-Lure and without dry ice, were positioned at the border with Italy in parallel to ovitrap, i.e. controlled biweekly. BG-sentinel traps were positioned in gardens where the presence of *Ae. albopictus* had been detected by ovitrap or reported by residents. The average number of eggs in 10 ovitrap present in a radius of 50 meters around the BG sentinel was then

compared with the number of adults in the BG-sentinels. It resulted in low densities of eggs, i.e. between 15 and 50 eggs, BG-sentinels contained 0-2 adults of the tiger mosquito. Seeing the difference in price, managing and the risk of manumission between ovitrap and BG-sentinels, the latter were abandoned for presence/absence purposes at low densities in the survey. During the same period sticky traps were tried out, but they were more difficult to operate. Ovitrap with a piece of polystyrene were tested as well. In this case, hatching of eggs was observed on it because of water capillarity, and determination of eggs was considered easier on the wooden stick in a large-scale system. Following these observations, ovitrap with the wooden paddle remained the main tool for the extensive survey system.

Whether the number of eggs present on ovitrap is correlated to the real presence of *Ae. albopictus* adults in a territory is often under discussion [41] but eggs on ovitrap can describe the presence of this mosquito correctly. In addition ovitrap are more reliable than other traps, such the adult trap BG-sentinel, to detect the mosquito presence at low densities as described above. The reduced cost in producing and checking ovitrap, permits the use of numerous traps in the survey. Even if cost-benefit at low densities of mosquito favours the use of ovitrap, a comparison with adults catches and number of breeding sites should be applied in a survey system. The density of eggs in ovitrap gives an indication of relative mosquito densities in an area compared to another one, which may represent an important indication of a problem related to the integrated control measures. Often neighbouring municipalities with the mosquito present over their whole territory show different egg averages on their ovitrap. This is an indication for the GLZ that there was a problem, such as treatments in catch basins not done with the planned schedule or bad information to residents in the municipality with higher densities. Despite these limits, mean number of eggs on ovitrap is still extensively used in surveillance systems.

Ae. albopictus spread could not be stopped, but adopted control measures managed to control or reduce the densities of the mosquito. The direct effect of a single type of control action was difficult to estimate, because in the system no control sites (untreated sites) were foreseen in non-managed municipalities. Usually if the mosquito disturbed the population the municipality and the citizens themselves reacted spontaneously adopting the suggested control measures. One example is the municipality of Melano (Mendrisiotto district), where the maximum egg densities of the season, i.e. end-August, increased from 65 ± 15 eggs/ovitrap in 2010, to 131 ± 30 in 2011, to 133 ± 32 in 2012 but decreased to 71 ± 23 in 2013.

The active participation of the population in managing breeding sites, joined with regular treatments of public catch basins are the key measures to control *Ae. albopictus*. This could be further improved in preparing specific educational programmes addressed to schools. For example in the surveillance system of Catalonia (Spain) a strong weight is given in educating the younger generations on the biology and breeding sites of *Ae. albopictus* (<http://elmosquitigrealescola.blogspot.com.es/>; <http://www.mosquitigregirona.cat>). This strategy, that involves children and their relatives, may engage the population deeply and sustainably in prevention. *Ae. albopictus* is a very annoying insect, biting at day-time and repeatedly, for this reason residents usually took into consideration the control measures suggested by the GLZ just after being directly concerned by the nuisance. Most of the contact to the GLZ came from residents suffering from the presence of the tiger mosquito. Citizens can obtain information on *Ae. albopictus* through the media and there is a web page dedicated to the thematic, but still the personal contact with an expert able to explain the particular case is considered important by the residents. Contact via email is rapid, however, a lot of people, in particular elderly individuals, prefer phone calls in order to delve into the subject. This service offered by the GLZ is time consuming, but has a durable effect and brings appreciation for the entire work. Mosquito densities detected in the BG-sentinel traps after phone calls to the GLZ in the peak season are similar to the ones reported in Italy [42], but in Ticino traps were positioned around houses of residents complaining about nuisance and not randomly on a surveyed area.

The implementation of the survey on tiger mosquito (3rd period) showed that its seasonal activity in Canton Ticino fits with what has been observed so far in Northern Italy, e.g. on <http://www.zanzaratigreonline.it>. In fact there was no activity in the winter period, the mosquito breeds in urbanized areas and the choice of the municipalities to be involved in the surveillance system corresponds with citizen reports.

In general, it is important to keep the control on the typology of breeding sites to be effective in preventing and controlling the establishment of the tiger mosquito. In fact new types of breeding sites can be discovered. This is what happened for covered oil sorters and flooded basements, as for tanks, broken fountains and cisterns. Because of the large amounts of water (>200 l) they had not been considered so far as breeding sites for *Ae. albopictus*: in fact, control of breeding sites usually concentrates on small water containers [35,36]. Tiger mosquitoes seem to breed in such containers if their surface is covered either by the structure of the container itself or by vegetation. Our new observations of larger breeding sites should encourage the control of larger containers in the future.

The strategies adopted since 2000 allowed the stabilization of the system on a large scale and made the public gradually aware of its responsibility and capacity to fight *Ae. albopictus*. Our surveillance program took into account treatments of every place where *Ae. albopictus* was detected as well as the supply of information to all citizens on prevention and control measures. This is the reason why no area can act as control (untreated area) for the evaluation of our prevention and control measures. In an on-going survey, ovitraps were placed in the area of the Canton Ticino bordering with Italy and in Italian municipalities just over the border where no control measures are undertaken. First results show that densities of eggs on ovitraps are far lower in Ticino (Tobias Suter, personal communication).

Bti was chosen as larvicide because this microbial insecticide is selective for mosquitoes with none or light reported effect on non-target organisms [43,44]. *Bti* is therefore considered the most environmentally friendly larvicide, which is important in case of application by non professionals, such as residents or in case of difficult weather conditions (i.e. rain forecast). Limits of the use of *Bti* for extensive application are that it is not persistent and that it does not kill all juvenile stages of mosquito. On the other hand, applications of diflubenzuron, an IGR (Insect Growth Regulator) which inhibits synthesis and deposition of chitin and is characterized by a low acute toxicity against mammals and a good safety margin with respect to non-target organisms including fish and birds [45,46], have to be executed following correct dilutions and weather forecasts not to risk undesired contamination of water systems. Advantages of diflubenzuron are its persistence for about one month [32,33] and its effect on all juvenile stages of mosquitoes. Therefore treatments of catch basins during the summer season with *Bti* had to be repeated weekly, in order to interrupt the aquatic phase in day summer temperature of about 25°C, whereas diflubenzuron treatment occurred monthly. As adulticides, the pyrethroids permethrin and cypermethrin were used. These neurotoxins are more toxic against cold-blooded than against warm-blooded animals. Pyrethroids were used because of their knockdown effect (killed immediately, without sub lethal effects) and their persistence was estimated at about a few days in summer conditions. The insecticides for larvicide applications were restricted to *Bti* and diflubenzuron, because they are the only ones for mosquito control currently allowed on the Swiss territory. In the future, if registered in Switzerland, we might also apply Vectomax® (Valent BioSciences), a combination of *Bti* and *Lysinibacillus sphaericus*, which increases the persistence of the larvicide activity compared to *Bti* alone also against *Ae. albopictus* according to the manufacturer and would minimize impact on the environment in case

of misuse. In any case in Canton Ticino all the applications are under the supervision of the cantonal authority for protection of air, soil and water (Sezione della protezione dell'aria dell'acqua e del suolo, www.ti.ch/spaas).

The methodology adopted in Canton Ticino for the 1st and 2nd period is described in the Guidelines for surveillance in Switzerland on *Ae. albopictus* available, in French and Italian, on the web pages of the Federal Office of Public Health and on the one for the Environment [47]. In fact this methodology of the 1st period is the one currently applied, since 2013, for the monitoring of *Ae. albopictus* at a national level in Switzerland. This strategy allowed the recent detection of *Ae. albopictus* in 3 service stations North of the Alps along highways: 1) the service station "Gotthard", the first service station after the Gotthard tunnel on the highway coming from Canton Ticino, 2) the service station "Heidiland" on the highway leading to Austria and Germany and 3) the service station "Grauholz" on the highway crossing Switzerland from east westwards [48-51]. The GLZ applied the collected data to a model of suitability for the presence of the tiger mosquito in order to evaluate the risk of expansion in Switzerland [50]. This model indicated that North of the Alps on the Swiss Plateau, the region between Geneva to Basel, offered favourable climatic conditions for the settlement of the mosquito. Since *Ae. albopictus* was found close to Geneva in the frame of the French "Plan National anti-dissémination de la dengue et du chikungunya" [51,52] in 2012 and 2013 as well as close to the Swiss border in three locations on the highway in Germany in 2012 [53]. The GLZ currently provides assistance to local authorities in Geneva Canton to survey *Ae. albopictus*.

Cost factors may affect strategies applied for the surveillance and the control of the tiger mosquito [35,36]. In our case, the limited budget resources for salaries and materials, which reached at the beginning around 10,000 CHF/year and rose up to 8 folds in the 2nd period to 20 folds in the 3rd one, as well as the increasing amount of work, due to the spread of the tiger mosquito, prompted us to constantly rationalize the system and to outsource part of the work to the concerned municipalities during the 3rd period. Hence, during this period, despite the fact that we had to reduce the number of ovitraps and checking rounds, the strategy used allowed to keep an accurate vision of the densities on the territory facing the problem of the time-consuming and expensive reading of ovitrap labels.

Here, most mosquito samples were identified using morphological characteristics. The use of the MALDI-TOF MS technique [30] validated the identifications.

Ae. albopictus is now widely distributed, spread mainly through the used tyre trade. Its presence has been reported in the United States of America, Latin America, several Pacific and Indian Ocean islands and Europe

[54]. *Ae. albopictus* has been reported in at least 11 countries in Europe [55-58]. Almost every European country has at least one part of its territory surveyed for *Ae. albopictus*. In some cases only survey strategies are applied, in other countries, control measures are foreseen in case of an epidemic of mosquito-borne pathogens, and only few systems include systematic and coordinated control measures [57,58]. The noteworthy feature of the surveillance system described in this paper is the start in 2000 before *Ae. albopictus* appeared in the territory (which happened between 2003 and 2008) and has been maintained along the whole process of the invasion of the exotic mosquito. The Canton Ticino is a small region therefore covering the territory with a surveillance system was feasible, but it is also one of the regions in Europe where the pressure for introduction of the tiger mosquito was the highest, particularly because of the intense road traffic entering from infested areas of southern Europe. In addition, climatic conditions in this canton were favourable for the establishment of the mosquito. The dispersal of the tiger mosquito was inevitable, but eliminations of new foci during its first arrival (2nd period) were successful, which delayed its establishment (3rd period) and even then control measures had an effect on mosquito densities. The fact, that control measures (applications and choice of insecticides) were under the supervision of a centralised authority (here the GLZ) helped to keep appropriate procedures under control. We achieved all those results by constructing the surveillance system step by step, financial restrictions and constantly increasing work prompted us to reconsider and refine strategies every year. Usually in Northern Italy the colonization process was faster with the species spreading into the whole urbanized area in 3-4 years from the first detection (Dr Romeo Bellini personal communication). Facing the problem from its beginning and maintaining trained and qualified personnel permitted us to gain knowledge of the territory and the related problematic. In addition, and this is an important point, this situation gave the population time to become aware of the *Ae. albopictus* thematic and the control measures to adopt. Before the arrival of *Ae. albopictus* in the Canton Ticino, residents were not familiar with mosquito nuisance, but with time they became familiar with the problematic related to the tiger mosquito without panic or alarmism and without consequences on the touristic activity, which is economically important for the region. We are convinced that it was worth facing *Ae. albopictus* on its arrival and adopting immediate control measures, followed by regular control measures after its establishment. In fact, this strategy permitted avoiding the surprise effect of having the tiger mosquito spread all over the territory facing a crisis situation which is more difficult to manage and probably much more expensive.

The risk of disease transmission in continental Europe related to the presence of *Ae. albopictus* is no longer theoretical [59]. In fact, in the recent past a chikungunya outbreak occurred in Italy in 2007 [60], two indigenous cases were reported in metropolitan France in 2010 [61] and recently four cases of CHK locally-acquired infection in Montpellier, France were notified to WHO [62]. Similarly, autochthonous cases of dengue were described in metropolitan France [63] and Croatia [64,65]. Therefore, the European Centre for Disease Prevention and control has published detailed guidelines for the surveillance of the vector [35]. In Canton Ticino, an average of around 110 eggs/ovitraps were observed during the peak of activity. This number is far lower than the one considered, i.e. > 200 eggs/ovitraps, as the threshold for epidemic risk of chikungunya or dengue [66]. However, an action plan in case of autochthonous cases has been implemented by the GLZ in collaboration with the medical authorities of the Canton Ticino based on the one set in Emilia-Romagna (Italy) [58]. No case of local transmission has been reported so far in Ticino and no infected mosquito has been detected [25].

Conclusions

To conclude, the objectives we had in 2000 regarding nuisance, sanitary risk and control of the use of insecticides to minimize impact on the environment, are so far, thirteen years later, accomplished. In addition today authorities are prepared to face possible emergencies in a competent way based on experience and up-to-dated information. The *Ae. albopictus* surveillance in Ticino settled also the basis for the elaboration and implementation of the current extended surveillance at the national level in Switzerland [49].

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EF, PL and NP designed the research and analysed the data from 2000 to 2013, LE from 2008–2013 and MT from 2011 to 2013. EF performed research and collected the samples from 2000 to 2013, and LE from 2007 to 2013. EF wrote the manuscript and LE provided the figures. All authors revised and approved the final version.

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4.3 PAPER 3

Ricerca di nuove specie di zanzare in Ticino mediante analisi MALDI-TOF MS. Verifica della presenza di nuove zanzare esotiche sul territorio cantonale tramite il controllo a campione delle uova raccolte con la rete di monitoraggio per la zanzara tigre. Rapporto 2013-2014. 2015. Bellinzona: Dipartimento della sanità e socialità del Cantone Ticino; Ufficio federale dell'ambiente.

Gruppo cantonale di Lavoro Zanzare, and Fondazione Bolle di Magadino

Oviposition traps (ovitrap) are the main tools used in the survey on *Aedes albopictus* lead in Canton Ticino to detect the mosquito presence. The eggs are then morphologically identified in the laboratory. Other exotic mosquito species such as *Ae. japonicus* and *Ae. koreicus* are nowadays occurring in Europe (Medlock et al. 2012). These species lay their eggs in the oviposition traps used in the *Ae. albopictus* survey. A new tool, the Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry (MALDI-TOF MS) technique (Schaffner et al. 2014), was applied to rapidly identify and differentiate these other exotic species and to verify the accuracy of the morphological differentiation previously used.

The original report is in Italian (Annex 2)

Research on new mosquito species in Canton Ticino identified with the MALDI-TOF MS technique.

English summary: Gruppo cantonale Lotta alle Zanzare & Fondazione Bolle di Magadino (2015) Ricerca di nuove specie di zanzare in Ticino mediante analisi MALDI-TOF MS, report for the Federal Office for the Environment (contract 00.0303. PZ / N492-2249).

The *Aedes albopictus* survey in Canton Ticino was mainly based on ovitrap capture (Flacio et al. 2015). Other container-breeding mosquitoes can lay their eggs in ovitraps and their morphological differentiation is rather difficult (Schaffner et al. 2014). These mosquito species include exotic invasive species that recently appeared in Europe (Medlock et al. 2012), in particular *Ae. japonicus*, which is spreading northern of the Alps (Schaffner et al. 2009) and *Ae. koreicus* spreading in northern Italy (Capelli et al. 2011). Hatching of eggs allowed morphological differentiation between *Ae. albopictus* and *Ae. geniculatus*, two mosquito species laying eggs in ovitraps and frequently observed in Canton Ticino. As the controlled area increased, the hatching of eggs of all positive paddles became too time-consuming and new identification tools were needed. Recently, the Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry (MALDI-TOF MS) technique was developed in Switzerland to rapidly differentiate mosquito species through their eggs (Schaffner et al. 2014). In 2013, this technique, coupled with the morphological examination, was applied for the identification of mosquito eggs in the frame of the surveillance system in Canton Ticino. Two positive ovitraps of each municipality were randomly chosen for a total of 202 analysed ovitraps. This allowed confirmation of the morphological differentiation of *Ae. albopictus* from *Ae. geniculatus* eggs (Flacio et al. 2015) and to discover two new exotic species in Canton Ticino: *Ae. koreicus* and *Ae. cretinus* (Figure 1 and Table 1), this last species was known to have a limited distribution in Greece, Georgia and Turkey (Giatropoulos et al. 2012). In 2014, the study focused on the municipality of Giubiasco, where *Ae. cretinus* was observed, and in all the municipalities at the border region where *Ae. koreicus* was previously detected. In addition analyses were lead in the municipality of Gambarogno where one *Ae. japonicus* adult was collected by a resident. Altogether, a total of 4,440 eggs from 147 positive ovitraps were prepared for the

MALDI-TOF MS technique by the Gruppo cantonale di Lavoro Zanzare and sent to Mabritec AG (Riehen, Switzerland) for the spectrum production and the species identification. Two eggs were identified as belonging to *Ae. cretinus*, 22 to *Ae. koreicus* and 8 to *Ae. japonicus* (Figure 1 and Table 1).

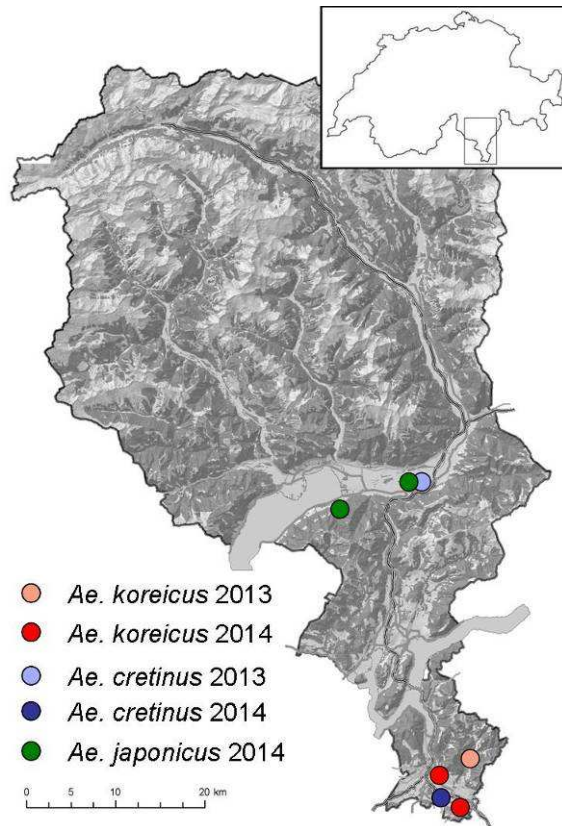


Figure 1. Location of the new exotic species observed between 2013 and 2014 with the MALDI-TOF MS technique

Table 1. Exotic mosquito species eggs identified with the MALDI-TOF MS technique in 2013 and 2014

Year	Species	Municipality	collection period	eggs analysed	LV03_East	LV03_North
2013	<i>Aedes cretinus</i>	Giubiasco	22.07-26.07	1	721071	115229
2013	<i>Aedes koreicus</i>	Breggia	19.08-23.08	2	723170	79484
2013	<i>Aedes koreicus</i>	Breggia	19.08-23.08	20	723170	79484
2014	<i>Aedes cretinus</i>	Balerna	07.07-11.07	1	721563	79186
2014	<i>Aedes cretinus</i>	Balerna	07.07-11.07	1	721647	78388
2014	<i>Aedes koreicus</i>	Coldrerio	23.06-27.06	15	720355	78869
2014	<i>Aedes koreicus</i>	Chiasso	21.07-25.07	3	723353	77504
2014	<i>Aedes koreicus</i>	Chiasso	15.09-19.09	4	722391	76183
2014	<i>Aedes japonicus</i>	Giubiasco	09.06-13.06	1	722005	115311
2014	<i>Aedes japonicus</i>	Gambarogno	04.08-08.08	1	706979	110178
2014	<i>Aedes japonicus</i>	Gambarogno	01.09-05.09	2	706965	110194
2014	<i>Aedes japonicus</i>	Gambarogno	15.09-19.09	4	706975	110210

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4.4 PAPER 4

Culicidae Fauna from Canton Ticino and Report of Three New Species for Switzerland. 2014. *Mitt. Schweiz. Entomol. Gesell.* 87: 163–82.

Flacio, Eleonora, Anya Rossi-Pedruzzi, Evelin Bernasconi-Casati, and Nicola Patocchi

The general mosquito fauna distribution in canton Ticino had to be updated. Knowing the distribution of indigenous mosquitoes was consider important as well to put the basis for further mosquito borne diseases transmission risk investigations.

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Culicidae fauna from Canton Ticino and report of three new species for Switzerland

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In The Canton of Ticino, a Swiss border region located on the Southern side of the Alps, knowledge on the mosquito fauna (Diptera: Culicidae) is both dated and incomplete. Therefore, we gathered data from different studies conducted between 2003 and 2011 on the mosquito fauna found in this area. Immature stages were sampled using a standard pint dipper (model 1132, BioQuip Products, USA). Adults were sampled using heavy duty EVS CO₂-baited mosquito traps (model 2801A, BioQuip Products, USA) with dry ice (CO₂) as an attractant, BG-sentinel traps (Biogents® AG, Regensburg, Germany) with BG-Lure (Biogents® AG) and CO₂ as attractants or Mosquito Magnet® Liberty plus with either Octenol or Lurex™ or Lurex3™ as attractant. A total of 27'688 mosquitoes (both juvenile and adult stages) were sampled in urban areas and major wetlands, with a focus on the Bolle di Magadino. Mosquitoes were morphologically identified to the species level. A total of 23 mosquito species were recorded, among which, three had never been found in Switzerland (*Aedes caspius*, *Coquillettidia buxtoni*, *Culex modestus*) and a further three which were new for the Canton of Ticino (*Ae. cataphylla*, *Cq. richiardii*, *Culex martinii*). Mosquito species causing major nuisance in settlements around wetlands are *Ae. sticticus*, *Ae. vexans* and *Cq. richiardii*, and in urban areas *Cx. pipiens / torrentium* and *Ae. albopictus*. Natural areas can act as a refuge for adult *Ae. albopictus* away from control measures in urban area, but they are unsuitable for reproduction. Wetlands in the Italian Province of Varese were sampled in order to compare mosquito fauna and anticipate introductions. No difference was detected except for the species *Uranotaenia unguiculata* that was not observed in the Canton of Ticino. Information on the mosquito fauna is important from a conservation perspective and also for our understanding of disease vector ecology

Keywords: Culicidae, surveillance, Bolle di Magadino, new records, *Aedes albopictus*, *Aedes caspius*, *Aedes cataphylla*, *Coquillettidia buxtoni*, *Coquillettidia richiardii*, *Culex martinii*, *Culex modestus*.

INTRODUCTION

Public attention on Culicidae (Diptera, Nematocera) is due to their nuisance and importance as vectors of pathogens. The possibility that allochthonous species are introduced into new areas due to climate change and worldwide trade, together with the discovery of their competence for new pathogens, underscore the need to improve our knowledge on mosquito distribution and diversity in specific regions. The climatic and geographic features of Ticino (Cotti *et al.* 1990), located south of the Alps in Switzerland, make this region of particular interest for studying the diversity of the mosquito fauna. The first published works on mosquitoes of Ticino were concerned mainly with malaria vectors, when, at the beginning of the last century, the

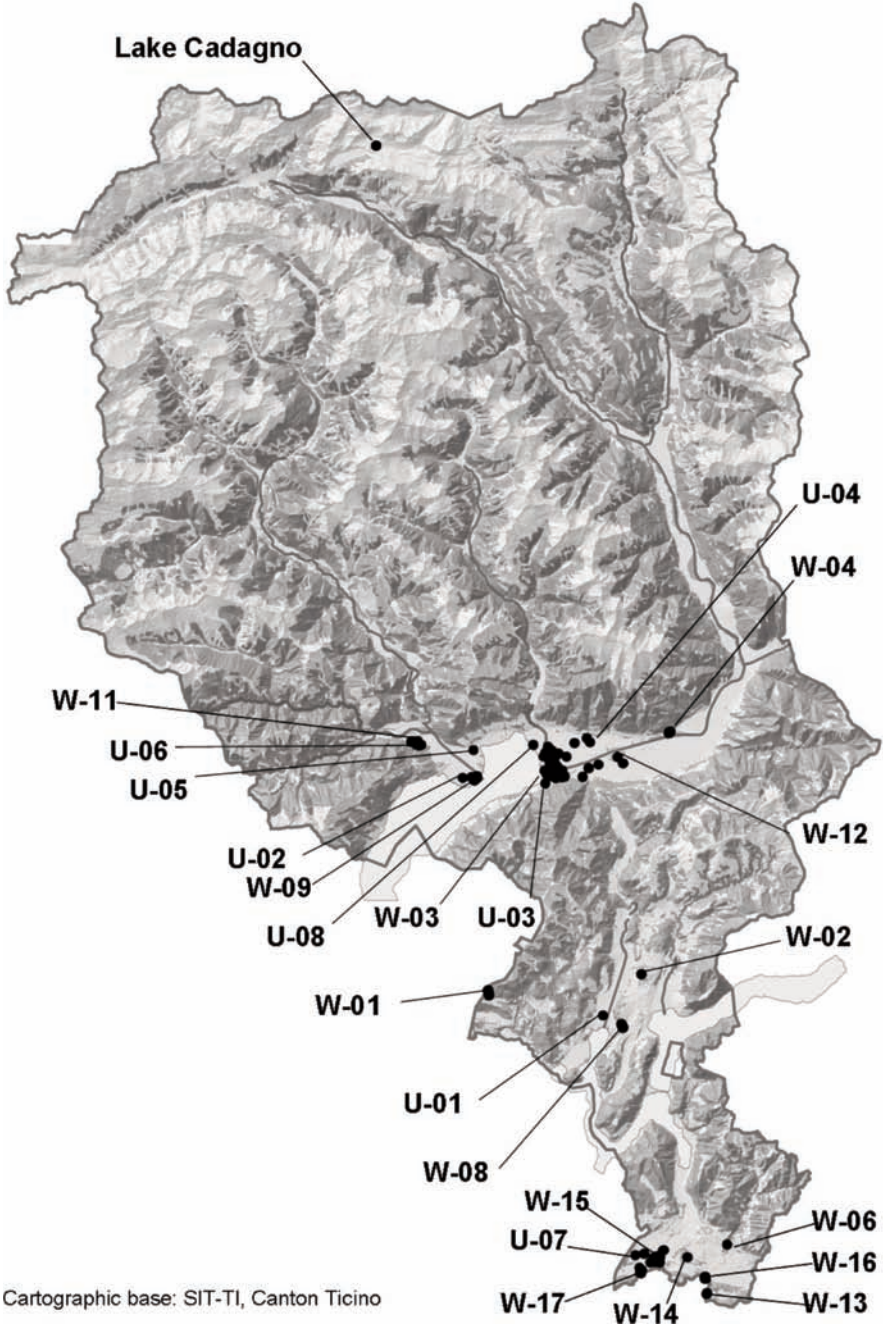


Fig. 1. Sampling locations of mosquitoes in canton Ticino (details are presented in Tab. 1).

Plane of Magadino and particularly the wetland reserve of the Bolle di Magadino, remained the only Swiss area still affected by the disease (Galli-Valerio 1905). Subsequently, malaria disappeared from the region in the 1930's (Borrani 1937). At the same time, another study on mosquitoes, not focused on malaria vectors, enlarged the knowledge of the mosquito fauna of the region (Vogel 1931).

Only at the end of the 1980's, when mosquito bites became a problem for residents and an economic threat for tourism for the region of Locarno, studies on mosquito fauna in the wetland area of Bolle di Magadino were reinitiated (Focarile 1987; Fouque *et al.* 1991) and mosquito control programmes were established (Fouque *et al.* 1998; Lüthy 1987).

Here we gathered data on mosquitoes from multiple sources covering a period between mid-April and the end of October 2003 to 2011. Monitoring at the Bolle di Magadino was intensified and additional wetlands of the Canton Ticino were investigated. To compare mosquito species and anticipate new introductions, wetlands in the Italian Province of Varese were also studied. In addition, mosquitoes were trapped to assess if they were a nuisance in urban areas and in villages bordering wetlands (Flacio 2004; Flacio & Rossi-Pedruzzi 2010; Rossi-Pedruzzi & Casati 2011). The recent urban spread of the tiger mosquito *Aedes albopictus*, which was observed for the first time in 2003 in Ticino (Flacio *et al.* 2004, 2010) pushed us to investigate if natural areas, surrounding colonized villages, could play a role

Tab. 1. Number of mosquito sampling sites and samplings according to the sampling locations in Canton Ticino (Switzerland) and Province of Varese (Italy) (2003 to 2011).

Area codes: W = wetland, U = urban zone.

Area ID	Sampling locations	N° sampling sites	N° of samplings					
			Project 1 2003	Project 2 2004	Project 2 2006	Project 3 2009	Project 3 2010	Project 4 2011
W-01	Astano	7				10	8	
W-02	Bolla di S. Martino (2512)	2				7	6	
W-03	Bolle di Magadino (2314, 2299)	122	118	49	31	75	65	22
W-04	Boschitt di Sementina (2302)	5				9	7	
W-05	Brabbia Swamp - Italy	19	36					
W-06	Gole della Breggia - Balerna	1					1	
W-07	Lake Biandronno - Italy	8	16					
W-08	Lake Muzzano (2323)	10				12	12	
W-09	Maggia river (2333)	12	4	7		9	7	2
W-10	Oasi della Bruschera - Italy	12	12					
W-11	Plane of Arbigio (2331, 3727)	17	15	24		8	13	
W-12	Plane of Magadino (2304, 2310)	7		5	3	2		
W-13	Swamps in Seseglio (2500)	7				12	9	
W-14	Valle della Motta - Coldrerio	1	1					
W-15	Wetlands of Genesterio (2503)	3	22	14		6	6	
W-16	Wetlands of Novazzano (2501)	5				6	7	
W-17	Wetlands of Stabio (2502, 2497)	31	20	43		11	12	
U-01	Agno	1		2				
U-02	Ascona	2	5			1		
U-03	Gambarogno	4		1		4	4	1
U-04	Lavertezzo	1		1				
U-05	Locarno	2	3			2		
U-06	Losone	2				1		
U-07	Stabio	2	7	5				
U-08	Tenero-Contra	2		1				

Note: The inventory number for wetlands of national or cantonal relevance is shown in parentheses.

in the development or the spread of the mosquito (Bernasconi 2010; Flacio & Rossi-Pedruzzi 2010). Gathering this information allows us to update the incomplete knowledge of the mosquito fauna in Ticino. This is important from a conservation perspective and for the assessment of risk hazards for human and animal health.

MATERIAL AND METHODS

Sampling locations

Data presented here originated from four projects conducted between 2003 and 2011. Project one (Flacio 2004) aimed at comparing the mosquito fauna in the major wetlands in Ticino and those in the Province of Varese (Italy) in order to anticipate new introductions and assess if mosquitoes breeding in wetlands could cause nuisance to inhabitants of surrounding villages. The second project continued the sampling in the Bolle di Magadino area as well as in the major wetlands in the south of Ticino: Stabio and Genesterio, in order to have a regular overview of the mosquito fauna in wetlands. The third project extended the survey to additional wetlands of the Canton that were closed to villages colonized by *Ae. albopictus* (Flacio *et al.* 2010) to see if wetlands could play a role in the geographic distribution of this species. The dispersal of *Ae. albopictus* in some wooded locations close to villages colonized by this species was also checked (Bernasconi 2010). The purpose of the fourth project (Rossi-Pedruzzi & Casati 2011) was to collect viruses in mosquitoes of some wetlands and urban zones, but faunistic information on the mosquito fauna of the wetland areas of the Bolle di Magadino and Maggia river was also considered. The sampling locations included in these four projects are listed in Tab. 1 and shown in Fig. 1.

In Ticino fourteen wetlands were controlled, among which eleven are protected (UFAM 2007; UNP 1993), as well as eight urban zones adjacent to the wetlands, i.e. residential locations outside the definite border of wetlands by the national or cantonal inventory. Urban zones were considered as close to wetland if the distance was <500m and distant if the distance was >500m (Tab. 2). A total of 246 sampling sites were investigated (Tab. 1), of which 122 were at Bolle di Magadino between mid-April and end of October 2003 to 2011. In all projects the sampling sites were visited three times in the season.

Additionally, mosquitoes were sampled in three protected wetland areas located in Italy in the bordering province of Varese, namely the Oasi della Bruschera (Protected area by regional laws n. 34934 of 19.07.1988), the Brabbia Ramsar swamp (Protection Area EUAP0323) and the lake Biandronno (Protection Area EUAP0308). Sampling (n=64) was conducted in the period from mid-June to the end of September 2003 (Tab. 1).

Sampling methods

Juvenile stages were sampled using a standard pint dipper (model 1132, BioQuip Products, Rancho Dominguez, USA), consisting of a white plastic container of 11 cm in diameter with a capacity of 350 ml. For every sampling site water collection was repeated at least 3 times in order to cover a total water surface of 30 m². Larvae were stored in 70 % ethanol after sampling whereas pupae were kept in the laboratory where they emerged as adults (Projects 1–3). For adult sampling, heavy duty EVS CO₂-baited mosquito traps (model 2801A, BioQuip Products) were placed with the

mosquito entrance at 1.5 m above ground level (projects 1–4) and dry ice (CO₂) was used as attractant. In order to extend the number of species, BG-sentinel traps (Biogents® AG, Regensburg, Germany) with BG-Lure (Biogents® AG) and CO₂ as attractants were placed on the ground (project 3–4). In some cases we used Mosquito Magnet® Liberty plus with either Octenol or Lurex™ or Lurex3™ as an attractant (www.mosquitomagnet.com) (project 2). Traps were placed in the afternoon and removed the following day no later than midday. For instant catches an insect net was used (project 1). Adults were killed by exposure to dry ice and stored at -20 °C.

Identification

Mosquitoes were identified to the species level using morphological keys (Becker *et al.* 2010; Romi *et al.* 1997; Schaffner *et al.* 2001; Severini *et al.* 2009; Stojanovich & Scott 1997). Identifications of species new to Switzerland were verified and confirmed by Dr. F. Schaffner (Institute of Parasitology, University of Zurich, Switzerland). When examining adults, only females were used.

Since differentiation of species by morphological characters is nearly impossible and molecular methods were not applied for the identification (Proft *et al.* 1999; Romi *et al.* 2000), we named *Anopheles maculipennis*: *An. maculipennis* sensu lato (s.l.). For the same reason the two biotypes of *Culex pipiens*, i.e. *pipiens* and *molestus* (Rudolf *et al.* 2013; Kang & Sim 2013), together with its sibling species *Cx. torrentium*, could not be characterized, therefore they are indicated as *Cx. pipiens / torrentium*. Similarly, *Aedes cinereus* and *Ae. geminus* could not be sorted and were considered as belonging to the *cinereus* group, here indicated as *Ae. cinereus / geminus*. Recently, changes were published within the tribe Aedini (Reinert 2000; Reinert *et al.* 2004) that led to a scientific debate and confusion since many names are used for a single taxon. In this paper, the traditional names are used, i.e. *Aedes* is considered as the genus, and *Ochlerotatus* and *Rusticoides* as subgenera for the *Ochlerotatus* spp. sensu Reinert (2000), the same with *Aedes (Finlaya) geniculatus* for *Ochlerotatus (Finlaya) geniculatus* sensu Reinert (2000), and with *Aedes (Stegomyia) albopictus* for *Stegomyia albopicta* sensu Reinert (2004).

RESULTS

A total of 27'688 individuals (9'293 larvae and 18'395 adults) were collected during 722/802 field samplings (Tab. 1). Three groups and twenty mosquito species were identified, six of which were new to Switzerland and/or Ticino.

New mosquito species for Switzerland and Ticino.

Three mosquito species new to Switzerland (*Aedes caspius*, *Coquillettidia buxtoni*, and *Cx. modestus*) and three to Ticino (*Ae. cataphylla*, *Cq. richiardii*, and *Cx. martinii*) are reported. Samples of these mosquito species are deposited in the collections of the Museo Cantonale di Storia Naturale of Lugano. Sampling locations and environment for these species in reference to the Swiss checklist (Briegel 1998; Merz *et al.* 2006) are compiled in Tab. 2, whereas detailed information including location, date, stage and sampling method are listed in Tab. 3. *Aedes (Ochlerotatus) caspius caspius* (Pallas, 1771) was collected at ten locations from the Italian border area to the region of the Plane of Magadino. This species was regularly recorded during the

Tab. 2. General overview and locations of mosquito species that occur in Canton Ticino (Switzerland) and Province of Varese (Italy).

Species	Reported from Switzerland literature	Reported from Canton Ticino literature	Sampling locations		Observed in		
			Canton Ticino (this study)	Province of Varese, Italy (this study)	wetlands	urban area close to wetland (<500 m)	urban area far from wetland (>500 m)
<i>Anopheles (Anopheles) claviger</i> (Meigen, 1804)	Briegel 1998	Borrani 1937; Briegel 1973; Fouque et al. 1991; Galli-Valerio 1905; Vogel 1931	W-01, W-04, W-13, W-16		x		
<i>Anopheles (Anopheles) maculipennis</i> Meigen, 1818	Merz et al. 2006	Borrani 1937; Briegel 1973; Focartile 1987; Fouque et al. 1991; Galli-Valerio 1905					
<i>Anopheles (Anopheles) maculipennis</i> s.l. Meigen, 1818	Briegel 1998		W-01, W-03-04, W-09, W-12-13, W-15-17, U-03, U-06-07	W-05, W-07, W-10	x		
<i>Anopheles (Anopheles) messeae</i> Falleroni, 1926	Merz et al. 2006	Briegel et al. 2002					
<i>Anopheles (Anopheles) plumbeus</i> Stephens, 1828	Briegel 1998	Borrani 1937; Briegel 1973; Focartile 1987; Vogel 1931	W-03-04, W-09, W-12, W-15-16		x		
<i>Aedes (Aedes) cinereus</i> Meigen, 1818	Briegel 1998	Focartile 1987; Fouque et al. 1991					
<i>Aedes (Aedes) cinereus / geminus</i>			W-01-03, W-08-09, W-11, W-15-17, U-06, U-08		x	x	
<i>Aedes (Aedes) geminus</i> Peus, 1970	dubious						
<i>Aedes (Aedimorphus) vexans vexans</i> (Meigen, 1830)	Briegel 1998	Fouque et al. 1991; Fouque et al. 1998; Lüthy 1987	W-02-04, W-06, W-09, W-11-13, W-15-17, U-01-04, U-08		x		
<i>Aedes (Finlaya) geniculatus</i> (Olivier, 1791)	Briegel 1998	Borrani 1937; Briegel 1973; Focartile 1987; Vogel 1931	W-01, W-03, W-11, W-16, U-08; surveillance on <i>Aedes albopictus</i>		x	x	x

Species	Reported from Switzerland literature	Reported from Canton Ticino literature	Sampling locations		Province of Varese, Italy (this study)	wetlands	Observed in	
			Canton Ticino (this study)	Province of Varese, Italy (this study)			urban area close to wetland (<500 m)	urban area far from wetland (>500 m)
<i>Aedes (Ochlerotatus) annulipes</i> (Meigen, 1830)	Briegel 1998	Fouque <i>et al.</i> 1991	W-03, W-17			x		
<i>Aedes (Ochlerotatus) cantans</i> (Meigen, 1818)	Briegel 1998	Focarile 1987; Fouque <i>et al.</i> 1991	W-01-03, W-08-09, W-11, W-15-17			x		
<i>Aedes (Ochlerotatus) caspius caspius</i> (Pallas, 1771)			W-03, W-08-09, W-11-12, W-15-17, U-02-03, U-07		W-05	x	x	x
<i>Aedes (Ochlerotatus) cataphylla</i> (Dyar, 1916)	Briegel 1998		Lake Cadagno					
<i>Aedes (Ochlerotatus) communis</i> (De Geer, 1776)	Briegel 1998	Briegel 1973						
<i>Aedes (Ochlerotatus) dorsalis</i> (Meigen, 1830)	Briegel 1998							
<i>Aedes (Ochlerotatus) excrucians</i> (Walker, 1856)	Briegel 1998							
<i>Aedes (Ochlerotatus) flavescens</i> (Müller, 1764)	Briegel 1998							
<i>Aedes (Ochlerotatus) intrudens</i> Dyar, 1919	Briegel 1998	Fouque <i>et al.</i> 1991	W-03-04, W-13, W-17			x		
<i>Aedes (Ochlerotatus) pulliatus</i> (Coquillett, 1904)	Briegel 1998	Borrani 1937						
<i>Aedes (Ochlerotatus) punctor</i> (Klaby, 1837)	Briegel 1998	Fouque <i>et al.</i> 1991						
<i>Aedes (Ochlerotatus) riparius</i> Dyar et Knab, 1907	dubious							
<i>Aedes (Ochlerotatus) sticticus</i> (Meigen, 1838)	Briegel 1998	Borrani 1937; Fouque <i>et al.</i> 1991	W-01-03, W-09, W-11-12, W-15, W-17, U-03-04, U-06-07			x	x	
<i>Aedes (Rusficoidius) refiki</i> (Medschid, 1928)	Briegel 1998							
<i>Aedes (Rusficoidius) rusticus</i> (Rossi, 1790)	Briegel 1998	Focarile 1987	W-09, W-15, U-02			x	x	
<i>Aedes (Stegomyia) albopictus</i> (Skuse, 1894)	Merz <i>et al.</i> 2006		W-16-17 and (Flacio <i>et al.</i> 2004)			x	x	x
<i>Coquillettidia (Coquillettidia) buxtoni</i> (Edwards, 1923)			W-03, W-08, W-11			x	x	
<i>Coquillettidia (Coquillettidia) richiardii</i> (Ficalbi, 1888)	Briegel 1998		W-03, W-08, W-11, U-06			x	x	

Species	Reported from Switzerland literature	Reported from Canton Ticino literature	Sampling locations		Observed in		
			Canton Ticino (this study)	Province of Varese, Italy (this study)	wetlands	urban area close to wetland (<500 m)	urban area far from wetland (>500 m)
<i>Culex (Barraudius) modestus</i> Ficalbi, 1890			W-09, W-13, W-15-17	W-05, W-10	x		
<i>Culex (Culex) pipiens</i> Linnaeus, 1758	Briegel 1998	Borrani 1937; Briegel 1973; Fouque et al. 1991; Lüthy 1987; Vogel 1931					
<i>Culex (Culex) pipiens / torrentium</i>			W-01-04, W-08-09, W-11-13, W-15-17, U-01-05, U-07, surveillance on <i>Aedes albopictus</i>	W-05, W-07, W-10	x	x	x
<i>Culex (Culex) Theobald, 1903</i>	dubious	Fouque et al. 1991					
<i>Culex (Culex) torrentium</i> Martini, 1925	Briegel 1998	Fouque et al. 1991					
<i>Culiseta (Malliotia) hortensis hortensis</i> Ficalbi, 1890	Briegel 1998	Fouque et al. 1991; Vogel 1931	W-01, W-03-04, W-08, W-11, W-13, W-15-17, surveillance on <i>Aedes albopictus</i>		x		x
<i>Culex (Neoculex) martini</i> Meischid, 1930	dubious		W-02-03, W-13, W-15,	W-05, W-07, W-10	x		
<i>Culex (Neoculex) territans</i> Walker, 1856	Briegel 1998	Focanile 1987; Fouque et al. 1991	W-02-04, W-08-09, W-11, W-13-16	W-05, W-07, W-10	x		
<i>Culiseta (Culiseta) fumipennis</i> (Stephens, 1825)	Briegel 1998						
<i>Culiseta (Culiseta) morsitans</i> (Theobald, 1901)	Briegel 1998						
<i>Culiseta (Culiseta) annulata</i> (Schränk, 1776)	Briegel 1998	Borrani 1937; Fouque et al. 1991	W-02-03, W-08-09, W-12-13, W-15-17, surveillance on <i>Aedes albopictus</i>	W-05, W-07, W-10	x	x	x
<i>Culiseta (Culiseta) glyptoptera</i> (Schiner, 1864)	dubious						
<i>Orthopodomyia pulchripalpis</i> (Rondani, 1872)	dubious						
<i>Uranotaenia (Pseudoficalbia) unguiculata</i> Edwards, 1913		Fouque et al. 1991		W-10			

survey from 2003 to 2011, with a total of 79 females and 31 larvae (representing 0.4 % of all mosquitoes recorded). This species was found as adults in both wetlands and urban environments. *Culex (Culex) modestus* Ficalbi, 1890 was observed in four different wetlands in Southern Ticino and in one close to Lake Maggiore. A total of 51 females and 5 larvae (0.2 % of the collected mosquitoes) were identified. This species was also recorded in the Province of Varese (Italy), with four *Cx. modestus* larvae collected at the Oasi della Bruschera and four additional larvae in the Brabbia swamp, representing 0.8 % of the total number of mosquitoes sampled in Italy. Finally, a total of 40 females belonging to the species *Cq. (Coquillettidia) buxtoni* (Edwards, 1923) (1.5 % of the collected mosquitoes) were recorded in three wetland ecosystems and in one urban location close to wetland.

In addition to these three mosquito species newly recorded in Switzerland, an additional three are new only to Ticino. One female of *Aedes (Ochlerotatus) cataphylla* Dyar, 1916 was collected at only one location, in the Saint Gotthard region, the Piora valley (close to the Lake Cadagno) at an altitude of 1960 meters above sea level. *Coquillettidia (Coq.) richiardii* (Ficalbi, 1889) specimens (620 adults; 2.25 % of the collected mosquitoes) were collected in four wetlands around the region of Magadino, in one near the Lake Muzzano and in an urbanized area close to a wetland ecosystem. Finally, 26 females and 88 larvae of *Culex (Neoculex) martinii* Medschid, 1930 (0.4 % of the collected mosquitoes) were identified in four wetlands from the Southern part of Ticino to the region of Magadino.

General overview of the mosquito fauna

Here, a total of 23 mosquito species were identified (Tab. 2). An overview of these mosquito species that occur in Ticino and in some wetlands in Italy in the bordering Province of Varese is provided in Tab. 2. All species that were recorded in Italy were also recorded in Ticino, except *Uranotaenia unguiculata*. In the following sections the mosquito fauna is presented according to the main environments.

a) Wetlands

The wetland area of Bolle di Magadino was an area of high sampling effort, because it is the major wetland area in Ticino and there is a fear amongst local people that mosquitoes could cause problems in residential areas. A total of 18 species could be recorded from this location (Tab. 4). In this ecosystem the mosquito density is influenced by the water level fluctuations of the Lago Maggiore that generate successive temporary flooded areas. The mosquito population is dominated by flood-water species like *Aedes vexans* and *Ae. sticticus*. In permanent water bodies, the most frequent species are *Anopheles maculipennis* s.l., *Ae. cinereus / geminus* and *Culex pipiens / torrentium*.

All species recorded in the other investigated wetland locations were present at Bolle di Magadino, except *Culex modestus* and *Aedes rusticus*, (Tab. 3). *Ae. rusticus* was recorded in the wetlands of Stabio and at the Maggia river.

b) Wetland border zones

A total of ten mosquito species whose main habitats are wetland areas were also collected in wetland border zones (Tab. 2). *Aedes albopictus*, the Asian tiger mosquito,

Tab. 3. Details on mosquito species newly identified in Canton Ticino and/or in Switzerland (part 1).

Species	Date	Sampling location	Method	Adults	Larvae
<i>Aedes albopictus</i>	28.7.2009	Ronchi - Muzzano	Insect net	4	
	1.9.2009	Wetlands of Novazzano	BG-Sentinel	1	
	20.7.2010	Wetlands of Novazzano	BG-Sentinel	1	
	28.8.2011	Val di Spinee - Vacallo	Insect net	10	
<i>Aedes caspius</i>	5.7.2003	Stabio	EVS CO ₂	10	
	5.7.2003	Wetlands of Stabio	EVS CO ₂	24	
	15.7.2003	Maggia river	EVS CO ₂	3	
	18.7.2003	Ascona	Insect net	2	
	16.7.2004	Plane of Arbigo	EVS CO ₂	8	
	3.8.2004	Gambarogno	EVS CO ₂	3	
	17.8.2004	Plane of Magadino	EVS CO ₂	2	
	27.8.2004	Bolle di Magadino	EVS CO ₂	5	
	28.8.2004	Plane of Magadino	EVS CO ₂	2	
	26.7.2006	Bolle di Magadino	EVS CO ₂ /Dipper	1	15
	3.8.2006	Bolle di Magadino	EVS CO ₂ /Dipper	1	15
	9.7.2009	Lake of Muzzano	EVS CO ₂	2	
	11.7.2009	Wetlands of Stabio	EVS CO ₂	6	
	21.7.2009	Wetlands of Novazzano	EVS CO ₂	1	
	24.7.2009	Gambarogno	EVS CO ₂	2	
	6.4.2010	Wetlands of Stabio	Dipper		1
	29.6.2010	Maggia river	EVS CO ₂	1	
	29.6.2010	Maggia river	BG-Sentinel	1	
	29.7.2010	Wetlands of Stabio	EVS CO ₂	1	
	6.7.2011	Bolle di Magadino	EVS CO ₂	1	
15.7.2011	Bolle di Magadino	EVS CO ₂	3		
<i>Aedes cataphylla</i>	1.06.2007	Lake Cadagno	Insect net	1	
<i>Coquillettidia buxtoni</i>	16.7.2004	Plane of Arbigo	EVS CO ₂	1	
	9.7.2009	Lake Muzzano	BG-Sentinel	3	
	9.7.2009	Lake Muzzano	EVS CO ₂	13	
	25.6.2010	Lake Muzzano	EVS CO ₂	1	
	25.6.2010	Lake Muzzano	BG-Sentinel	13	
	29.6.2010	Losone	BG-Sentinel	4	
	27.7.2010	Lake Muzzano	BG-Sentinel	3	
	15.7.2011	Bolle di Magadino	EVS CO ₂	2	

Tab. 3. Details on mosquito species newly identified in Canton Ticino and/or in Switzerland (part 2).

Species	Date	Sampling location	Method	Adults	Larvae
<i>Coquillettidia richiardii</i>	16.7.2004	Plane of Arbigo	EVS CO ₂	57	
	28.7.2004	Plane of Magadino	EVS CO ₂	22	
	9.7.2009	Lake Muzzano	BG-Sentinel	4	
	9.7.2009	Lake Muzzano	EVS CO ₂	4	
	17.7.2009	Losone	EVS CO ₂	61	
	17.7.2009	Losone	BG-Sentinel	85	
	17.7.2009	Maggia river	EVS CO ₂	2	
	23.7.2009	Bolle di Magadino	EVS CO ₂	1	
	25.8.2009	Bolle di Magadino	EVS CO ₂	6	
	28.8.2009	Plane of Arbigo	EVS CO ₂	2	
	28.8.2009	Losone	BG-Sentinel	6	
	5.8.2009	Plane of Magadino	EVS CO ₂	1	
	20.8.2009	Lake Muzzano	BG-Sentinel	1	
	16.7.2010	Bolle di Magadino	BG-Sentinel	2	
	29.6.2010	Plane of Arbigo	EVS CO ₂	13	
	29.6.2010	Losone	BG-Sentinel	238	
	29.6.2010	Maggia river	EVS CO ₂	1	
	14.7.2010	Plane of Magadino	EVS CO ₂	3	
	14.7.2010	Plane of Magadino	BG-Sentinel	1	
	16.7.2010	Plane of Magadino	EVS CO ₂	2	
	27.7.2010	Lake Muzzano	EVS CO ₂	1	
	27.7.2010	Lake Muzzano	BG-Sentinel	11	
	20.8.2010	Plane di Arbigo	EVS CO ₂	13	
	20.8.2010	Losone	BG-Sentinel	36	
	3.9.2010	Plane of Magadino	EVS CO ₂	2	
	3.9.2010	Bolle di Magadino	EVS CO ₂	4	
	6.7.2011	Bolle di Magadino	EVS CO ₂	1	
	6.7.2011	Bolle di Magadino	EVS CO ₂	2	
	15.7.2011	Bolle di Magadino	EVS CO ₂	12	
	5.8.2011	Bolle di Magadino	EVS CO ₂	5	
19.8.2011	Bolle di Magadino	EVS CO ₂	7		
26.8.2011	Bolle di Magadino	EVS CO ₂	13		
9.9.2011	Bolle di Magadino	BG-Sentinel	1		
<i>Culex martinii</i>	25.6.2003	Wetlands of Genesterio	EVS CO ₂ /Dipper	15	40
	26.9.2003	Wetlands of Genesterio	Dipper		20
	16.7.2009	Bolla di S. Martino	BG-Sentinel	3	
	21.7.2009	Swamps in Seseglio	EVS CO ₂	1	
	23.7.2009	Bolle di Magadino	Dipper		2
	23.7.2009	Bolle di Magadino	Dipper		4
	23.7.2009	Bolle di Magadino	BG-Sentinel	7	
	27.7.2009	Bolle di Magadino	Dipper		16
	31.8.2009	Swamps in Seseglio	Dipper		1
	6.10.2009	Bolle di Magadino	Dipper		5
<i>Culex modestus</i>	5.7.2003	Wetlands of Genesterio	EVS CO ₂	1	
	15.7.2003	Maggia river	EVS CO ₂	13	
	7.8.2003	Wetlands of Stabio	Dipper		5
	11.7.2009	Wetlands of Stabio	BG-Sentinel	2	
	11.7.2009	Wetlands of Stabio	EVS CO ₂	5	
	21.7.2009	Swamps in Seseglio	BG-Sentinel	1	
	21.7.2009	Swamps in Seseglio	EVS CO ₂	27	
	20.7.2010	Wetlands of Novazzano	EVS CO ₂	1	
29.7.2010	Wetlands of Genesterio	EVS CO ₂	1		

was the only urban species encountered in these wetland border zones. Adults of this species were recorded at low densities in some natural locations bordering urban areas. This included a wooded area in Muzzano connecting the hard shoulder of the highway to the town, in the wood of the Val de Spinee crossing Morbio Inferiore and Vacallo, and in the wood of the wetland Prà Coltello, which is close to the industrial area of Novazzano (Tab. 2). No evidence of reproduction in larval habitats was observed in these areas and no *Ae. albopictus* were found at Bolle di Magadino. For the malaria vector species *Anopheles maculipennis* s.l., only two adults were collected in an urban area of the Plane of Arbigo and three close to the Bolle di Magadino, while 74 larvae were sampled in a pound in the town of Stabio.

c) Urban areas

In urban areas, six mosquito species were recorded (Tab. 2). Thanks to a surveillance system on *Aedes albopictus* (Flacio *et al.* 2013) it is known that *Culex pipiens / torrentium* and *Ae. albopictus* were the most abundant. Both species are container-inhabiting species but the first was the most frequently found, in particular in catch basins and water storage containers. *Ae. geniculatus* eggs are frequently sampled together with those of *Ae. albopictus* in the framework of the surveillance system of this species that is based on ovitraps placed in urban environments.

Seasonal dynamic of mosquitoes

An overview of the seasonal dynamics of the mosquito species in the surveyed locations is presented in Tab. 5. Early in the season, from April till the end of May, when the water temperature is still low, larvae belonging to the species *Aedes cantans*, *Ae. caspius*, *Ae. cinereus / geminus*, *Ae. sticticus*, *Ae. vexans*, *Anopheles maculipennis* s.l., *Culex hortensis*, *Cx. pipiens / torrentium*, *Cx. territans* and *Culiseta annulata* were found. All species were collected during summertime. In autumn, only adults belonging to the species *Aedes cinereus / geminus*, *Ae. intrudens*, *Ae. sticticus*, *Ae. vexans*, *Culex pipiens / torrentium*, *Anopheles plumbeus* and *An. claviger* could be collected.

DISCUSSION

During the different surveys, we collected mosquitoes belonging to 23 species among which six were new to Switzerland and/or the Canton of Ticino. In doing so, we have provided valuable contributions to our knowledge on the mosquito fauna of the region. The invasive species *Aedes albopictus*, spreading from Italy to Mediterranean countries, is now established in Ticino and the population density is continuously increasing (Flacio *et al.* 2013). Our results show that *Ae. caspius*, a species newly reported for Ticino, is well established in the area. It is a Palaearctic species, known all over Europe (Snow & Ramsdale 1999) and considered to be one of the most nuisance causing mosquito species in northern Italy. It is considered as a brackish water species but can develop in fresh water areas such as rice fields, ponds, marshes and ditches. The female bites all warm-blooded animals and humans. They bite during the day, at dusk and dawn, usually outside the home. Adults can fly long distances, sometimes over 20 km from their breeding sites. The species is considered as a vector for West Nile virus (WNV), Tahyna virus, the bacterium *Francisella tularensis* (the agent of tularaemia) and agents of animal filariasis (Becker 2010). In

Tab. 4. Number of mosquito species collected at Bolle di Magadino (2003-2011) according to their developmental stage (larvae and adults) and number of samplings.

Species	Adults	Samplings	Larvae	Samplings
<i>Aedes annulipes</i>	3	3	0	0
<i>Aedes cantans</i>	366	31	44	3
<i>Aedes caspius caspius</i>	10	7	15	2
<i>Aedes cinereus / geminus</i>	313	84	150	28
<i>Aedes geniculatus</i>	2	2	0	0
<i>Aedes intrudens</i>	25	16	0	0
<i>Aedes sticticus</i>	1139	64	668	38
<i>Aedes vexans vexans</i>	1503	108	1353	67
<i>Anopheles claviger</i>	16	5	0	0
<i>Anopheles maculipennis</i> s.l.	207	53	571	79
<i>Anopheles plumbeus</i>	32	20	65	3
<i>Coquillettidia buxtoni</i>	2	1	0	0
<i>Coquillettidia richiardii</i>	54	25	0	0
<i>Culex hortensis hortensis</i>	0	0	15	4
<i>Culex martinii</i>	7	1	27	4
<i>Culex pipiens / torrentium</i>	392	90	358	31
<i>Culex territans</i>	14	2	1052	73
<i>Culiseta annulata</i>	15	8	91	10

2011 Schaffner & Mathis (2011) also reported the presence of this species in Switzerland, although the location was not specified. However, it is surprising that *Ae. caspius* has not been described earlier in this country since it occurs all over Europe. This species is well known in Italy (Veronesi *et al.* 2012) mainly in wetlands such as rice growing areas. Here, we observed *Ae. caspius* development in one Italian wetland, the Brabbia swamp, where 100 larvae were collected. Even if *Ae. caspius* was detected both in wetlands and urban areas, the observed densities of this mosquito species limit the risk of disease transmission and disturbance of local residents.

The habitat of *Culex modestus* in Ticino and Italy was restricted to wetland ecosystems. Only a limited number of specimens were collected. Its occurrence between July and August corresponds to the findings of a previous survey in Italy (Veronesi *et al.* 2012). This Palaearctic species can be observed all over Europe, except in the Northern countries of Scandinavia and the Baltic Sea (Schaffner *et al.* 2001). In Italy this species is particularly present along Northern coastal regions (Severini *et al.* 2009). The females are very aggressive toward humans and they usually bite at night. *Cx. modestus* is a known vector of WNV and suspected vector

of other arboviruses, such as Sinbis, Tahyna, and Lednice (Lundström 1999; Balenghien *et al.* 2007). Although this species demonstrates a high vector competence in some contexts, it does not represent a major health threat in Ticino due to its rare occurrence.

In this study, adults of *Coquillettidia buxtoni*, observed in association with *Cq. richiardii*, were restricted to three wetland locations. Larvae and pupae of this Palaearctic-Mediterranean species develop in permanent waters where they are attached to the roots of the aquatic plants such as sweet flag (*Acorus* spp., very rare in Ticino) and bulrushes (*Typha* spp.) for breathing. They could not be collected in our projects. *Coquillettidia buxtoni* is reported in France, Italy, Romania and Spain (Ramsdale & Snow 2001). This species lives in association with *Cq. richiardii* (Severini *et al.* 2009), as observed in our study. Females bite humans and animals outdoors. There is no knowledge about its arboviral vector competence (Schaffner *et al.* 2001) and control measures against larvae are difficult to implement.

Although we recorded *Coquillettidia richiardii* for the first time in Ticino, this species seems to be widespread in all wetlands around the region of Locarno and the Lake of Muzzano. The absence of previous records in Ticino may be due to the fact that, in previous surveys, traps for adults were not used. Capture of *Cq. richiardii* adults is almost the only method to obtain indication of the presence of this species, and its nuisance was probably confused with one of the species considered the most abundant, i.e. *Aedes vexans*. Females are active between the end of June and the beginning of September and can be a problem for local residents. *Cq. richiardii* is a wetland inhabiting Euro-Siberian species, distributed all over Europe (Ramsdale & Snow 2001). Its ecology is similar to that of *Cq. buxtoni*. The females bite humans and are potential vectors of Batai, Tahyna and WNV. This species posed a nuisance to only one urban settlement, a campsite close to the plane of Arbigo, therefore we do not think there is the potential for arboviral transmission.

Although *Aedes cataphylla* has not been previously described in Ticino, the presence of one individual collected at high altitude is not surprising. This North-Holarctic species can be observed all over Europe and is usually found in cold climates (Schaffner *et al.* 2001). Larvae hatch in depressions filled by snow-melted water and the females bite humans. No pathogen transmission is reported for this species.

Finally, a few individuals of *Culex martinii*, a species newly reported for Ticino, were collected from the end of June to the beginning of October, mostly as larvae. Briegel (1998) in the Swiss checklist for Culicidae stated that the presence of this species in the country needed confirmation, but Schaffner & Mathis (2011) mentioned two observations as well (locations not specified by the authors). Our work confirms the presence of this species in Ticino. This Palaearctic-Mediterranean species is mostly distributed in Central and Southern Europe (Snow & Ramsdale 1999). *Cx. martinii* takes blood meals on batrachians and therefore easily entered the adult traps used. No role of transmission of infectious agents to humans has been reported.

Mosquitoes found in the major wetland of Ticino: the Bolle di Magadino

Many of our study sites were located in the wetland of Bolle di Magadino. Some mosquito species identified here confirmed previous records from this wetland

(Focarile 1987; Fouque *et al.* 1991). This is the case for *Anopheles claviger*, *An. maculipennis* s.l., *An. plumbeus*, *Aedes cinereus* / *geminus*, *Ae. vexans*, *Ae. annulipes*, *Ae. cantans*, *Ae. geniculatus*, *Ae. intrudens*, *Ae. sticticus*, *Culex pipiens* / *torrentium*, *Cx. hortensis*, *Cx. territans* and *Culiseta annulata*. We could not confirm the presence of *Ae. rusticus* in this location, which was reported by Focarile (1987), despite the large number of specimens (17'256 adults and 6'317 larvae) collected. Interestingly, the currently most represented species, i.e. *Ae. vexans* and *Ae. sticticus*, were not mentioned in Focarile's study (1987). Similarly, Fouque *et al.* (1991) reported the presence of *Ae. punctor*, *Cx. theileri* and *Orthopodomyia pulchripalpis*, three species that we did not capture. Briegel (1998) considered that *Cx. theileri* and *Or. pulchripalpis* needed confirmation, which could not be provided here.

Fouque *et al.* (1998) assessed that *Aedes vexans* was responsible for most of the nuisance at the Bolle di Magadino, but we collected almost as many *Ae. sticticus* as *Ae. vexans* and both species frequently bite humans. The detection of *Ae. caspius*, *Culex martinii*, *Coquillettidia buxtoni* and *Cq. richiardii* for the first time at Bolle di Magadino may be due to the large amount of sampling and the first use of EVS CO₂-baited traps, which allow *Coquillettidia* species to be easily captured.

Environments of mosquitoes

All the mosquitoes collected in the wetlands are representative of this ecosystem. We observed that a few species such as *Anopheles maculipennis* s.l., *Aedes cinereus* / *geminus*, *Ae. geniculatus*, *Ae. caspius*, *Ae. vexans*, *Ae. sticticus*, *Culex pipiens* / *torrentium*, *Culiseta annulata*, *Coquillettidia buxtoni* and *Cq. richiardii* disperse from the wetlands to more residential areas causing some disturbances to the inhabitants. *Ae. vexans* and *Ae. sticticus* are the most disturbing species around the Bolle di Magadino and the level of nuisance is strongly related to the efficacy of the larval treatments that are used every year in the reserve (Guidi *et al.* 2011; Lüthy 2001; Lüthy & Patocchi 2013). Like Briegel *et al.* (2002), we observed that the presence of malaria vectors like *An. maculipennis* s.l. and *An. plumbeus* is too low to represent a risk for malaria transmission both in wetlands and in surrounding urban areas. *An. plumbeus* is confined to the wetlands and only 5 adults of *An. maculipennis* s.l. could be collected around the wetlands. The genus *Coquillettidia* causes nuisance in a camping area close to the Plane of Arbigo. It is difficult to reduce their numbers with larvicides, but further studies in this direction should be encouraged. *Cx. pipiens* / *torrentium* can be considered as a ubiquitous species. Currently, two forms (or biotypes) are recognized (Farajollahi *et al.* 2011) i.e. *Cx. pipiens* form *pipiens*, colonizing mostly natural environments, and *Cx. pipiens* form *molestus*, present generally in urban habitats. These two subspecies are characterized by different feeding behaviours: mostly birds for biotype *pipiens* and mammals for biotype *molestus*. *Cx. pipiens* s.l. is considered a bridge vector for several arboviruses, for example WNV (Andreadis 2012). Therefore the monitoring and the virus analysis of this species are included in an ongoing project conducted in Ticino (O. Engler, pers. comm.). The densities of the other mosquito species are too low to cause real nuisance.

Aedes albopictus, as a neobiota, represents a special case with its potential vectorial competence (Gjenero-Margan *et al.* 2011; Gould *et al.* 2010; Moutailler *et al.* 2009; Schmidt-Chanasit *et al.* 2010; Talbalaghi *et al.* 2010; Vazeille *et al.* 2008). This species was discovered in Ticino for the first time in 2003 (Flacio *et al.* 2004).

It is known to be a container-inhabiting urban mosquito colonizing the Mediterranean area of Europe. *Ae. albopictus* has colonized the majority of the urban locations surveyed with the monitoring system established in Ticino since 2000 (Flacio *et al.* 2013). There was no evidence that this species is reproducing in the forest areas of Ticino (Bernasconi 2010; Flacio & Rossi-Pedruzzi 2010). In the sampling locations no potential habitats like tree holes filled with water or neglected human containers were found. Therefore, we assume that adults use the fresh wooden areas to rest. As most efforts to control *Ae. albopictus* are implemented in urbanized parts, individuals can take refuge in surrounding natural areas. Data on the seasonal dynamics of *Ae. albopictus* show that the first hatching of overwintering eggs occurs around mid-April, while their population densities peak around mid-August and adults can be captured until mid-November (Flacio *et al.* 2013).

Concerning the seasonal dynamic of the other mosquito species, our observations fit to what is known. In some cases the number of individuals was so limited i.e. for *Aedes annulipes* (four adults) and *Ae. rusticus* (five adults) that we cannot comment on their seasonal dynamic.

The different projects here add new species to the known Swiss Culicidae fauna and improve our knowledge of the fauna of Ticino. In the future, additional exotic species could be introduced into Ticino, for instance *Aedes japonicus* coming from north of the Alps where it was recently recorded (Schaffner *et al.* 2009; Schaffner *et al.* 2011) or *Ae. koreicus* from Italy (Capelli *et al.* 2011). Other European species new to Switzerland could be detected such as *Culiseta subochrea* which was observed close to Geneva (Direction Générale de la Nature et du Paysage, Etat de Genève, Anya Rossi-Pedruzzi, pers. comm.). In Ticino most studies focus on sites located at low altitudes where human activity is concentrated and the risk of virus transmission to humans is highest. However, mosquito captures at higher altitudes have been reported by Schaffner & Mathis (2011). Observations reported here should encourage new ongoing studies on various aspects of mosquitoes not only in Ticino but also elsewhere in Switzerland.

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5 DISCUSSION

5. DISCUSSION

In 2000, in Canton Ticino, the knowledge on mosquito was scarce. Some faunistic investigations related to malaria and control measures had been undertaken against wetland mosquitoes causing nuisance in some residence areas. A more accurate evaluation on mosquito species in Canton Ticino was needed to assess the biodiversity in order to control mosquitoes that produce nuisance to the population. In addition, the recent arrival of exotic invasive species, like *Ae. albopictus*, in the nearby Italy in the 90's prompted us to evaluate the importation risk of such mosquitoes, which are vectors of several exotic pathogens, in Ticino.

5.1 MOSQUITO SPECIES AND THEIR DISTRIBUTION

Thanks to the combination of different sampling methodologies the knowledge on the diversity of mosquito species and on their geographic distribution in Canton Ticino improved. New European native mosquito species were discovered (Annex 3): *Ae. caspius*, *Ae. cataphylla*, *Cx. martinii*, *Cx. modestus*, *Cq. buxtoni* and *Cq. richiardii* (Flacio et al. 2014), among them *Ae. caspius*, *Cx. modestus* and *Oc. buxtoni* were reported for the first time in Switzerland (Briegel 1998, Merz et al. 2006). *Cx. martinii* presence on the Swiss territory was considered dubious (Briegel 1998), probably because the larva morphology is closed to *Cx. territans* and because *Cx. martinii* is rare in central Europe (Schaffner and Mathis 2013). Although the survey concentrated on wetlands on the valley floor, *Ae. cataphylla*, a mountainous species, was collected occasionally outside the surveyed areas. We did not investigate mountainous regions, which may explain why we could not confirm the presence of the snow-mosquitoes *Ae. communis* (Briegel 1973) and *Ae. pullatus* (Borrani 1937). Schaffner and Mathis (2013) who investigated high altitudes could not confirm their presence either. Similarly, despite several samplings at the Bolle di Magadino, we were not able to collect the two species that needed confirmation (Briegel 1998), previously reported by Fouque et al. (1991a), i.e. *Cx. theileri* and *Or. pulchripalpis*. Likewise *Ae. punctor* was not confirmed in the area, but this species seems to be very scarce at the national level (Schaffner and Mathis 2013).

Most mosquito species were recorded in wetlands or wooded areas (Annex 3) and they have only occasionally a nuisance impact on the surrounding urbanized areas (Flacio et al. 2014). Two floodwater mosquitoes, very aggressive towards humans and displaying a high dispersal ability, i.e. *Ae. vexans* and *Ae. sticticus*, may cause extreme nuisance to villages around the Bolle di Magadino in the absence of control measures. Our survey showed that the Bolle di Magadino, the main natural reserve, displays the largest diversity with 18 mosquito species recorded (Flacio et al. 2014).

Differently, *Cx. pipiens*, with its two biotypes, i.e. *Cx. pipiens pipiens*, that mainly colonizes natural environments and feeds on birds, and *Cx. pipiens molestus*, that is present mainly in urban habitats and feeds on mammals (Farajollahi et al. 2011) is ubiquitous. In fact, *Cx. pipiens* is the most representative mosquito species in residential areas jointly with the exotic species *Ae. albopictus*.

The survey performed in nearby Italian wetlands (Flacio et al. 2014) aimed to check if new species were imported in Canton Ticino. Mosquito species in the Italian areas were represented as well in the Swiss wetlands, with the exception of *Ur. unguiculata*. This species does not bite humans nor other mammals and, even if a new strain of WNV has been isolated from this species, no evidence of pathogenicity has been demonstrated (Pachler et al. 2014).

5.2 AEADES ALBOPICTUS AND OTHER EXOTIC MOSQUITO SPECIES

We started the surveillance system of *Ae. albopictus* in Canton Ticino in 2000. It aimed to adopt preventive actions against this mosquito which had rapidly spread in Italy (Romi et al. 1999). *Ae. albopictus* was detected in Switzerland for the first time in 2003 (Flacio et al. 2004). This exotic species is so far the most successful in invading large European areas and is still spreading. Different introduction pathways have been reported in Europe. The international trade of used tires introduced the mosquito in the Veneto region in Italy in 1991 (Romi 1996b), in France in 1999 (Schaffner and Karch 2000), in Belgium in 2000 (Schaffner et al. 2004) and in the Netherlands in 2010 (Scholte et al. 2010). In this last case the mosquito was also introduced in greenhouses via the trade of Lucky bamboo, an

ornamental plant (Scholte et al. 2007). The tiger mosquito spread can then be attributed to ground public and private transport (Medlock et al. 2012). Road axes are favouring its dispersion from the Mediterranean basin northward. Currently the tiger mosquito is established in most costal Mediterranean regions: starting westward from the Spanish region of Alicante and reaching eastward Greece (Figure 10). The entire Italian territory is affected by this mosquito and the species was reported, but not considered established, in many other regions in Europe, like central and north France, Belgium, south Germany, the Netherlands, Czech Republic, Slovakia, Austria, Serbia, Bulgaria, Romania, Turkey and on the Black Sea in Russia. A total of twenty-one European countries have reported the presence of the tiger mosquito (VBORNET 2015).

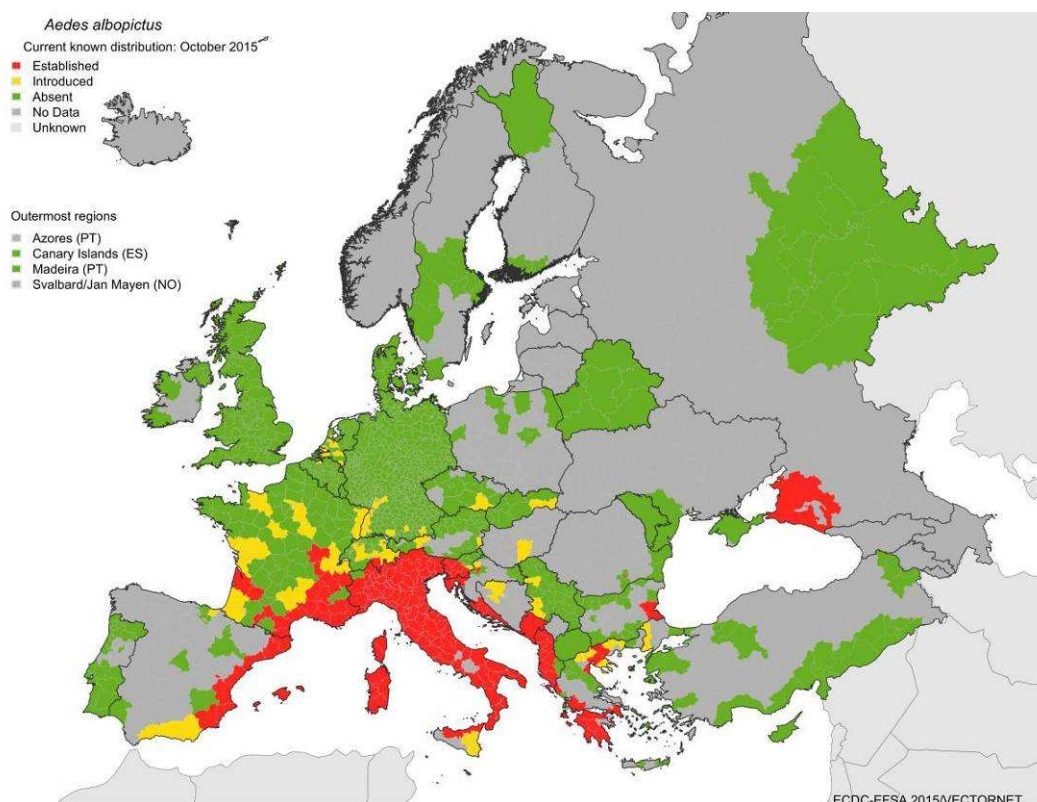


Figure 10. European *Ae. albopictus* distribution (status October 2015) (http://www.ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx)

In Switzerland, the tiger mosquito was introduced, not through international trade of used tires but thanks to road traffic (See paper 1, 2015, Medlock et al. 2012). It is established south of the Alps (See paper 1) and has been recently reported north of the Alps in 2013

and 2014 (Müller et al. 2013, 2015). In Canton Ticino, we observed that introductions of the mosquito were first related to the intense road traffic on the highway E35 from Italy (See paper 1). Later, the presence of the mosquito in the Italian border facilitated its arrival and establishment in urban areas in the Mendrisio district, in the south border region. From there, the spread followed a gradient moving northwards. Once established, the mosquito increased its spread through most valley floor areas thanks to local traffic and active flight with a clear progression (See paper 1). *Ae. albopictus* is nowadays well established and is active from mid-April until mid-November, without winter activity (See paper 1).

We mostly concentrated the *Ae. albopictus* survey on urban areas because of the risk of disease transmission to human and because this mosquito is considered in Europe container breeding colonizing human settlements (European Centre for Disease Prevention and Control 2012). However, we also screened tree holes and general waste in natural environments for the presence of *Ae. albopictus* larvae, without success. Only adults were captured and only in woods close to urbanized areas where the mosquito was established. This suggests that woods represent refuges for the mosquito. Therefore it is strongly recommended to avoid any breeding place for *Ae. albopictus* in woods by keeping them clean from any waste, like small containers, used tires etc. This is the only control measure. In fact, in these habitats adulticide cannot be applied. Recent observations reported its presence in forested areas in the Italian Province of Como and in the Mendrisio district (Suter et al. 2016). Therefore it cannot be excluded that *Ae. albopictus* could extend its colonization to natural environment in the future. Interestingly *Ae. japonicus*, a species close to *Ae. albopictus*, is now widely spread north of the Alps (Schaffner et al. 2009, Werner and Kampen 2013) mainly in forested areas in tree holes or containers (Kaufman and Fonseca 2014, Medlock et al. 2015). *Ae. japonicus* spread in these areas is a warning for the potential spread of *Ae. albopictus* in these environments. However, currently, it remains unclear if the tiger mosquito will ever be able to establish north of the Alps. This mosquito requires a mean annual temperature, varying, according to authors, over 5° C (Benedict et al. 2007) or over 11° C (European Centre for Disease Prevention and Control 2009) for adult survival, a summer temperature around 25-30° C

for the development (Straetemans 2008) and a mean winter temperature, also varying according to authors, $>-1.8^{\circ}\text{C}$ (Benedict et al. 2007), $> 0^{\circ}\text{C}$ (Knudsen et al. 1996, Medlock et al. 2006) or $> -1^{\circ}\text{C}$ (Neteler et al. 2013) for overwintering of eggs. Laboratory studies showed that a soil temperature threshold at -10°C for 12 hours is required for survival of overwintering eggs of European *Ae. albopictus* strains (Thomas et al. 2012). In general Canton Ticino weather conditions are favourable for *Ae. albopictus* spread and establishment, which widely occurred in the South part, whereas in the North and at altitude above 400 m its presence is still sporadic probably because of lower temperature and road traffic (See paper 1). Maps produced from the ECDC (European Centre for Disease Prevention and Control 2009, 2013) predict, in the context of climate changes in a long term scenario (2030), that the suitability for *Ae. albopictus* establishment will move towards north-east covering whole central Europe and reaching the coastal areas of Baltic countries.

During our study period other container-breeding exotic invasive mosquito species were detected in Europe: *Ae. aegypti*, *Ae. atropalpus*, *Ae. japonicus*, *Ae. koreicus* and *Ae. triseriatus* (Medlock et al. 2012). Similarly, in Ticino, additional exotic species were identified in ovitraps: *Ae. koreicus*, *Ae. japonicus* and *Ae. cretinus* (Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015, Suter et al. 2015) using MALDI-TOF MS analysis. This technique was applied because of the difficulties in differentiating eggs of these species morphologically (Schaffner et al. 2014). In addition morphological differentiation of adults of these exotic species can be undertaken only by skilled entomologists and even though the differentiation between *Ae. albopictus* and *Ae. cretinus* remains difficult. Therefore the real distribution of these species in the Canton is still unclear and further investigations are required. *Ae. japonicus* adults were first observed in 2014 in the Locarno and Bellinzona districts (Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015). Eggs of *Ae. koreicus*, a species previously reported in Italy (Capelli et al. 2011, Montarsi et al. 2013), were detected at the border in the Mendrisio district in 2013 and 2014 (Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015, Suter et al. 2015). *Ae. cretinus* was detected in the Bellinzona and Mendrisio districts in

2013 and 2014, respectively (Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015). *Ae. cretinus* is an exotic species but is not considered invasive. Until now this species was reported only in Greece, Georgia and Turkey (Becker et al. 2003, Giatropoulos et al. 2012). Knowledge on its biology is scarce and *Ae. cretinus* is not considered of medical importance due to its rarity (Schaffner, Geoffroy, et al. 2001). *Ae. cretinus* can hybridize with *Ae. albopictus* in laboratory producing sterile hybrid eggs and *Ae. cretinus* females can easier crossmate, therefore it is likely that *Ae. albopictus* presence would negatively affect *Ae. cretinus* populations (Giatropoulos et al. 2014).

All these invasive exotic species arrived in Europe mainly via trade of used tires and further spread by ground road transports through public and private transports. *Ae. japonicus* is currently wide spread north of the Alps (Schaffner et al. 2009, Werner and Kampen 2013, Kampen and Werner 2014) and Ticino attracts many tourists originating from these areas, therefore tourists may have imported this mosquito with their private transport, that is to say *Ae. japonicus* probably reached Canton Ticino from northern regions. *Ae. koreicus* recently invaded northern Italy (Capelli et al. 2011, Montarsi et al. 2013). Its introduction in Ticino might have used the same pathway as *Ae. albopictus*. Finally *Ae. cretinus*, as mentioned above, might have been confused with *Ae. albopictus* for years, therefore presumably both species were introduced jointly. The establishment of these exotic species threatens human and animal health because of their potential involvement in transmission of pathogens of tropical and subtropical origin (Medlock et al. 2012).

Globally, further studies are needed to understand the real distribution of these exotic species in Canton Ticino mainly because of the similarity in their morphology and behaviour with *Ae. albopictus* (aggressive to human during daytime and container breeding). Their distribution might in fact be underestimated both in Canton Ticino and other countries. All these species in Canton Ticino were observed in parallel with *Ae. albopictus* in urbanized areas, but it is also known that *Ae. japonicus* rather prefers natural environments, such as bushy and forested areas (Medlock et al. 2015). *Ae. japonicus* and *Ae. koreicus*, in addition, seem to resist to lower temperatures than *Ae. albopictus* (Medlock et

al. 2015), therefore they could colonize higher altitudes. They also seem to start activity earlier than *Ae. albopictus* (Medlock et al. 2015). This could increase the general nuisance due to exotic mosquitoes in Canton Ticino. Nevertheless *Ae. albopictus* remains so far the most representative exotic species in our area (Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015).

5.3 MOSQUITO SAMPLING

One purpose of this work was to detect the maximum of mosquito species, therefore we used different trap models available on the market, different attractants and different sample methodologies, i.e. collecting eggs, larvae or adults, which were applied simultaneously.

Ovitrap with the wooden paddle as support for egg deposition were used to detect the presence of *Ae. albopictus* along all the surveys. The wooden paddle was preferred as support for eggs to others, such as germination paper or piece of polystyrene (European Centre for Disease Prevention and Control 2012), because they were considered as more practical for not skilled collaborators, that is to say municipality workers (Flacio et al. 2015).

Focks (Focks 2003) discussed the reliability of ovitraps as indicators of *Ae. albopictus* densities, but Carrieri and his group observed a correlation among mean number of eggs in ovitraps and adult densities (Carrieri et al. 2012). Considering ovitraps that remained on the field from 2006 to 2014 we evidenced that the number of eggs does not show a normal distribution (See paper 1), which should be taken into consideration once survey results are compared with plain averages. We adopted a generalised linear mixed model with a negative binomial link function to investigate the relationship between egg densities not normally distributed and covariates such as time (month and year). The trend of growing egg densities over the years and the seasonal peak in August is similar to the one that will result with calculation with plain averages, but the numbers are more accurate.

5 Discussion

In any case according to our results ovitraps detect the presence of the tiger mosquito faster as other survey tools and are practical from an economic and managing point of view for an extensive survey programme.

The arrival of new exotic invasive species occurring in Europe (Medlock et al. 2012, 2015) complicates the identification of mosquito species collected by ovitraps. In fact their eggs cannot be morphologically differentiated from the ones of *Ae. albopictus*. The distinction is possible only after hatching or by the MALDI-TOF MS analysis of the eggs (Schaffner et al. 2014). Egg hatching was already occasionally applied to confirm the morphological differentiation between *Ae. albopictus* and *Ae. geniculatus* eggs (Zamburlini and Frilli 2003), because both species can be observed in the ovitraps. The MALDI-TOF MS analysis confirmed that our morphological differentiation of these species was reliable (Flacio et al. 2015). The MALDI-TOF MS technique also allowed the detection of other exotic species, i.e. *Ae. koreicus*, *Ae. japonicus* and *Ae. cretinus* (Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015). The detection of new species decreases the accuracy of ovitraps for *Ae. albopictus* and means that other survey tools have to be applied in parallel with ovitraps in further studies in Canton Ticino.

Larval sampling gives good information of the origin of the adults therefore larvae were always sampled in parallel to adults when origin of nuisance caused by wetlands mosquitoes was tested. Searching for larvae allowed identification of new breeding sites for *Ae. albopictus* (Flacio et al. 2015) such as underground tanks, flooded basements of building yards and other recipients containing thousands of litres of water like tanks, broken fountains and cisterns. This differs from what was reported in the literature so far (Hawley 1988, Paupy et al. 2009, European Centre for Disease Prevention and Control 2012), that is to say that *Ae. albopictus* breeds in containers with less than 200 l of water. When *Ae. albopictus* breeds in large amount of water, the place has to be somehow closed either covered by vegetation or underneath the ground. *Ae. albopictus* larvae, in fact, were never detected in open surfaces such as abandoned swimming pools. *Ae. albopictus* production in these large breeding sites is pretty high, hordes of adults were observed flying out from underground tanks, and infested flooded basements affected the entire

neighbourhood. Reproduction in small containers simulates the natural breeding sites, such as bamboo stumps, bromeliads and tree holes, available in Asiatic forested areas, the place of origin of the tiger mosquito. Small breeding sites give a protection effect, which can be provided by larger ones if they are somehow closed. This might be one explanation of the discovery of these new breeding sites. Another one could be the extreme ecological adaptation characteristic of this mosquito species (Paupy et al. 2009). This indicates that one should constantly explore the environment to identify *Ae. albopictus* new breeding sites and should not consider only what was reported in the literature so far.

For adult collections, we used different traps that simulated host presence: the breathing with carbon dioxide and body odours with different products. We used three types of traps: EVS CO₂-baited mosquito, Mosquito Magnet® Liberty plus and BG-sentinel, and different attractants, such as CO₂, Octenol, Lurex™ and BG-Lure, because their capacity in collecting different species depends on how and what kind of odour they release (Vythilingam et al. 1992, Becker et al. 1995, Miller et al. 2005, Irish et al. 2008, Li et al. 2010, Cilek et al. 2011, Kline et al. 2012), on their form (Hoel et al. 2007, Brown et al. 2008, Xue et al. 2008, Drago et al. 2012, Roiz et al. 2012, Lühken et al. 2014) and on the height at which they are positioned (DiMenna et al. 2006). Hence the accumulation of the different characteristics enlarges the spectrum of mosquito species that can be collected.

BG-sentinel traps were applied to collect in particular *Ae. albopictus* adults, because they have been designed to be specific for dengue vectors, such as *Ae. albopictus* and *Ae. aegypti*. We used them mainly to detect *Ae. albopictus* in natural environments but we did not use them on a regular basis, because they detect the presence of *Ae. albopictus* later than ovitraps (Flacio et al. 2015), they are more expensive and at more risk of manumission than ovitraps. Therefore, they were mainly used to reduce adult nuisance for residents. The CDC Gravid Trap (model 1712) was not used to survey *Ae. albopictus* because it collects a restricted spectrum of species, i.e. mostly *Cx. pipiens* (White et al. 2009, Lühken et al. 2014) but it was used, as well as BG-sentinel traps in another study that investigated virus in mosquitoes (Engler et al. 2013).

Other traps, such as sticky traps, in which the adult remains attached to a viscous surface (Facchinelli et al. 2007) that are used in some surveys on *Ae. albopictus*, were not applied because they were considered too demanding to be used in an extensive survey program. Insect net or human landing collection were occasionally used, mostly to enhance adult captures or to make an immediate determination of nuisance species when required.

Nowadays a new developed trap for gravid *Aedes* species, the BG-GAT trap (Biogents AG, Regensburg, Germany) is available. This trap attracts ovipositioning *Aedes* females simply with water, form of the trap and ovipositioning cues. CO₂ supply is not required, nor electricity and the insects are not damaged in the trap, because they do not have to cross a fan. The Zumpa Mosquito trap (ISCA Technologies, St Spring, Unites States) is another new trap, which uses CO₂ and SkinLure™ as attractants that shows interesting results in *Ae. albopictus* and *Cx. pipiens* capture (Bhalala and Arias 2009). For future investigation the use of these new tools should be considered.

5.4 MOSQUITO CONTROL MEASURES

In Canton Ticino, mosquito control measures are undertaken since years to limit the nuisance to residents and tourists and to decrease the risk of disease transmission (Lüthy and Patocchi 2014, Flacio et al. 2015)(Annex 3).

In this work, we assessed if wetland mosquitoes were causing nuisance to the surrounding villages and it came out that mosquitoes were remaining confined in the natural areas of origin (Flacio et al. 2014). This means that control measures against wetland mosquitoes are effective. In particular the ones undergoing over 25 years in the Bolle di Magadino, which uses granules containing *Bacillus thuringiensis israelensis* (VectoBac G®) against larvae (Lüthy and Patocchi 2014). In fact around the '80 of last century villages closed to the Bolle di Magadino, as well as tourists, suffered from mosquito nuisance,(Lüthy 1987). Our investigations showed that the main mosquitoes responsible for the nuisance were *Ae. vexans* and *Ae. sticticus* (Flacio et al. 2014) and not only *Ae. vexans* as previously reported (Fouque et al. 1991, 1998). Another species, *Cq. richiardii*, also causes some nuisance but control measures are difficult, because larvae of this genus remain under water level

attached to aquatic plants and do not get in contact with the biological larvicide, (VectoBac G®). Therefore other control strategies should be investigated and adapted to its biology such as water level drawdown as suggested by Batzer and Resh (Batzer and Resh 1992).

The evaluation of combined control measures for urban environment is more difficult (Flacio et al. 2015). It remains unclear which control measures - information to the population, active removal of breeding sites, treatments of catch basin in public and/or in private areas - has the major impact. The adopted surveillance system did not permit to leave untreated areas (control areas) to assess the efficacy of our strategies. Nevertheless comparing data among municipalities helped to identify gaps in the surveillance system, such as incorrect scheduled treatments or incorrect information on mosquito control to the residents. Recently, a parallel study in Mendrisio district and in bordering Italian areas showed a 2.26 fold higher tiger mosquito presence in the Italian areas where no surveillance system is undertaken compared to Mendrisio (Suter et al. 2016). When the tiger mosquito was present only in restricted areas, targeted control measures with larvicides and adulticides managed to eliminate the mosquito for years, i.e. its presence could not be detected with ovitraps. This is not surprising, it is known that coordinated control measures succeed in eliminating the mosquito, for example in used tire deposits in northern France (Schaffner and Karch 2000), in a port in New Zealand (Holder et al. 2010). In the Italian island Sardinia the mosquito was found in used tires deposits, eliminated but reappeared in 2006, ten years later and is now established (Cristo et al. 2006). This is also the case of a used tire deposit in Belgium, where the mosquito reappeared thirteen years later (Boukraa et al. 2013). Lucky bamboos were the cause of the mosquito introduction in the Netherlands in 2005 (Scholte et al. 2007). Afterwards the mosquito was found as well in used tires deposits, but interventions prevented its establishment so far (Stroo et al. 2014). Hence apparently, the mosquito can be eliminated but it often reappears. However, we believe it is worth to have control measures against the mosquito because delaying the nuisance advantages the resident quality of life. In addition, this delay can be useful to improve the surveillance system, because usually authorities are convinced of the importance of a surveillance system once the mosquito is introduced in an area.

5 Discussion

The main difficulty to control mosquito in Canton Ticino is related to the urban environment structures: urban settlements are mostly characterised by two story houses, surrounded with private gardens providing breeding sites, like small containers and small catch basins. Vegetation is used as resting place for adults, and inhabitants and their domestic animals offer a source of blood for mosquito reproduction. So, if not controlled, the mosquito can easily spread from house to house and rapidly colonize a whole village. Therefore involvement of the population was considered a key point in the surveillance system. Residents are directly interested in reducing the mosquito densities, due to its extreme nuisance: numerous itching bites, and its activity in the morning hours and late afternoon. The major difficulty in the involvement of the population is that usually residents are retro active: they react once they are personally concerned by the nuisance, mainly in late summer, and only few residents adopt the preventive recommendations of avoiding breeding sites in spring already, when *Ae. albopictus* starts its activity. Hence mosquito control measures are less effective. However, if all actors of the surveillance system properly apply control measures, the mosquito presence can even remain unnoticed because of very low density. This is the case in the main park in Lugano, an important tourist attraction during summer where *Ae. albopictus* was detected in 2008, where control measures were applied and where mosquito adults are no more disturbing.

Although the control of the tiger mosquito is difficult and its spread in Ticino could not be prevented, the information campaign and the control measures undertaken by municipalities managed to maintain a low mosquito density and in some cases even decreased mosquito densities at the municipality level. These results are probably the maximum we could expect. Complete *Ae. albopictus* elimination from the territory is not feasible, due to favourable weather conditions (See paper 1) and a complex dynamic of continuous introduction from abroad as well as local spread. The presence of an effective surveillance system to control this annoying mosquito and potential vector of pathogens is necessary and is reassuring residents, tourists and media. This also allows a better coexistence of residents and tourists with *Ae. albopictus*.

5.5 RISK OF PATHOGEN TRANSMISSION

The context of mosquito-borne diseases potentially affecting Europe has drastically changed since 2000, when this work started. In fact, new vector competences have been discovered and mosquito-borne diseases that once were considered rare in Europe are now occurring regularly.

Malaria was considered eradicated in Europe, but re-emerged in Greece between 2009 and 2013 with some autochthonous cases (Hellenic Centre for Disease Control & Prevention 2013). WNV, transported by migratory birds, which in 1999 caused a large epidemic in New York and spread rapidly over the USA in the subsequent years (Kilpatrick AM. 2011), caused sporadic cases within Europe by the end of last century (Hubálek and Halouzka 1999) and is now widely circulating in Europe (Calzolari et al. 2012, Engler et al. 2013, Sambri et al. 2013, Pachler et al. 2014). Similarly, Usutu virus, also transported by migratory birds, is widely spreading along Europe (Ashraf et al. 2015). *Ae. albopictus* became an efficient vector for chikungunya virus (CHKV) thanks to a mutation of an amino acid of the virus (Tsetsarkin et al. 2007). This is the cause of the large CHK epidemic that occurred between 2005 and 2006 in the South West Indian Ocean Islands, in particular in the Reunion Island where 34% of the total island population was concerned (Pialoux et al. 2007). *Ae. albopictus* was later shown to transmit CHKV in temperate regions during an outbreak of 200 cases in Italy (Province of Ravenna) in 2007 (Angelini et al. 2007). The virus had been introduced by a citizen returning from India. Indigenous CHK cases were reported in metropolitan France in 2010 (Grandadam et al. 2011) and in 2014 (Delisle et al. 2015). In addition, dengue cases were reported in 2010, 2013 and 2015 in France (La Ruche et al. 2010, Marchand et al. 2013, Institut de veille sanitaire 2015) and in 2010 in Croatia (Schmidt-Chanasit et al. 2010, Gjenero-Margan et al. 2011). Global movements of persons favour the introduction of arboviruses (Weaver and Reisen 2010). The important epidemic of CHK in the Caribbean islands that started in fall 2013 in the island of Saint Martin (Cassadou et al. 2014) and spread rapidly all over the region (Mowatt and Jackson 2014) favoured the increase of imported CHK cases in Europe in 2014 (European Centre for

Disease Prevention and Control 2014, Paty et al. 2014, Delisle et al. 2015). Currently *Ae. albopictus* is a vector of CHKV, DENV, ZIKV (zika virus) as well as dirofilaria worms and has been reported to be able to transmit 27 viruses under laboratory conditions (Paupy et al. 2009, Ayres 2016) and European strains of this species have shown high efficiency to transmit CHKV and dengue virus (Vega-Rua et al. 2013). The risk of arbovirus transmission in Europe related to *Ae. albopictus* is real. European authorities for disease prevention, like ECDC, suggest to apply surveillance and control systems where the mosquito is present as well as preventive measures where there is a potential for its arrival (European Centre for Disease Prevention and Control 2012). ECDC also encourages anticipatory measures in case of local virus transmission as well as reports of infected patients.

In Canton Ticino a map of mosquito species distribution is now available (See paper 1, 2015, Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015). Several mosquito species detected in the Canton may be vectors of animal or human pathogens (Annex 3). Recorded densities of mosquitoes that could act as vectors are low and these mosquitoes seem to be restricted to wetlands. Hence the risk of disease transmission can be considered as low. In urban settlements the most representative mosquito species are *Cx. pipiens* and *Ae. albopictus*, whereas the presence of *Ae. geniculatus*, *Ae. japonicus* and *Ae. koreicus* can be still considered as rare. In contrast, the current distribution and density of *Ae. cretinus* remain unclear due to the difficulty in differentiating this species from *Ae. albopictus*.

Arboviruses were not investigated in the present study but another study screening adult mosquitoes sampled in the Bolle di Magadino in 2006 for arboviruses showed absence of any arbovirus that could affect animal or human health (Flacio et al. 2007). Further studies on mosquitoes demonstrated the presence of USUV in *Cx. pipiens/torrentium* species (Engler et al. 2013). Previously, local bird experts (Roberto Lardelli personal communication) observed circumscribed blackbird deaths without clear ethiology, but viruses like USUV or WNV were suspected.

In 2013 the GLZ was mandated by the Swiss Federal Office for Health (BAG) to assess a strategy for preventing local transmission of arboviruses. The GLZ adopted a strategy similar to the one applied in Emilia Romagna after the CHK outbreak in 2007 (Carrieri et al. 2011). This strategy includes that the medical system has to take into consideration symptoms of CHK and dengue after return from an endemic area. The Cantonal Office for Health collects confirmed cases (with patient privacy guarantee), evaluates sites where the patient spent time during infectivity period, and alerts the GLZ on these areas. The GLZ evaluates if control measures (larvicide and adulticide applications under the GLZ supervision) have to be adopted, based on data collected on ovitraps. This action plan contains several shortcomings, for example mild symptoms confused with a summer flu, difficulty to trace with precision the patient displacements during their infectivity, evaluation of *Ae. albopictus* egg densities. So far, the GLZ undertook several interventions in areas where affected persons with CHK or dengue spent their time and no local spread of these diseases was recorded. Trying to keep mosquito densities at low level reduces not only the nuisance, but also the possibility of a rapid and wide spread of diseases.

Knowledge on mosquito species present in a territory has to be continuously updated in parallel with investigations on arboviruses. In fact, all actors of the transmission cycles are involved in a complex dynamic that includes, among others, Europe global transport of goods, global movements of people or bird migrations in a context of climate change favouring import of pathogens in non-endemic areas.

6 CONCLUSIONS

6. CONCLUSIONS

In Canton Ticino the fact that a group of scientists, the GLZ, was active, dealing with mosquito problematic, even if restricted to wetland mosquitoes of the Bolle di Magadino, greatly helped to create a larger network to anticipate the introduction of invasive species in 2000. In fact, the GLZ promoted mosquito surveys from a local level to a national level to face different scientific and political aspects related to these insects. Our work greatly increased the knowledge on the mosquito fauna and distribution in wetlands and urban areas in Canton Ticino, which allowed assessments on nuisance, risk of mosquito-borne diseases and establishment of appropriate control measures. In addition the detection of exotic mosquitoes on the territory opened new investigations on their ecology and vector competence.

The main topic of this thesis was the surveillance system of *Ae. albopictus*, which started in 2000 and is still on-going. At the beginning very few was known on how to proceed, in fact we were pioneer in Europe. Before 2000, the tiger mosquito had been detected in Albania (Adhami and Reiter 1998), Italy (Romi et al. 1999) and in northern France (Schaffner and Karch 2000). Only few surveillance systems were active at that time despite the observation of *Ae. albopictus* in Europe and its rapid spread in Italy: some surveillance systems in the affected Italian areas, some preliminary surveys on the French Mediterranean coast and a national survey on used tires deposit in France (Schaffner, Boulétreau, et al. 2001). Therefore we proceeded step by step creating a methodology that could fit as best as possible our territory characteristics and financial and political restrictions. It took time to get the surveillance recognized at institutional level. If *Ae. albopictus* was first detected in 2003 (Flacio et al. 2004) Cantonal authorities recognized the need of a surveillance with a governmental resolution only in 2008. This included surveillance of *Ae. albopictus* by the GLZ and expanded the competence of the GLZ on mosquitoes over the whole canton. This was done in collaboration with the cantonal Institute for Microbiology (ICM) and since 2016 with the Laboratory of Applied Microbiology (LMA) (University of Applied Sciences and Arts of Southern Switzerland, SUPSI). The major improvement in the surveillance occurred in 2009 when the

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municipalities were involved in the survey and control activities, which permitted to extend the survey and to reach residents more efficiently. Currently, the GLZ is continuously improving the surveillance, facing the establishment of *Ae. albopictus* in the territory.

The surveillance system adopted in Canton Ticino is now considered as the base for any activity on *Ae. albopictus* at the Swiss level. In 2011 the Federal Office of Environment (BAFU) and the Federal Office of Health (BAG) adopted our surveillance system for a national concept on *Ae. albopictus* control (Bundesamt für Umwelt and Bundesamt für Gesundheit 2011). A study financed by BAFU (Biebinger 2013) considered the surveillance system of *Ae. albopictus* in Canton Ticino as an example for other Swiss Cantons (Laboratorio microbiologia applicata SUPSI 2015).

Furthermore *Ae. albopictus* data collected in Canton Ticino were used to model the potential establishment of this mosquito in other parts of Switzerland in the context of global warming (Neteler et al. 2013). This model predicted that the Lake of Geneva and, to some extent, the Swiss Plateau are threatened by *Ae. albopictus*. This gives indication where surveillance priorities are to be applied, in addition to the Canton Ticino and the Swiss motorway network. In fact during late summer 2015 *Ae. albopictus* was detected in residential areas in both semi cantons of Basel (Müller 2016). In these areas weather conditions are very similar to the ones in the city of Freiburg in Germany, where recent records suggest that the mosquito managed to overwinter (KABS 2016). This has alerted the Swiss authorities about the real risk of *Ae. albopictus* establishment North of the Alps.

Our work also reported the presence of other exotic mosquito species in Canton Ticino, i.e. *Ae. cretinus*, *Ae. japonicus* and *Ae. koreicus*. Very few is known on their distribution, ecology and vector competence. This could be investigated in the framework of the Swiss Vector Entomology Group (SVEG) that includes all researchers and institutions dealing with vectors. SVEG also represents an excellent platform to coordinate all aspects regarding mosquitoes on the Swiss territory. Our results showed that Switzerland is no longer a country where mosquitoes can be considered as a side problem.

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8. ACKNOWLEDGEMENTS

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Thanks to the mosquitoes for being so fascinating ☺

9 ANNEX

9. ANNEX

9.1 ANNEX 1. Preparation of Mosquito Egg Samples for MALDI-TOF MS Identification

PREPARATION OF MOSQUITO EGG SAMPLES FOR MALDI-TOF MS IDENTIFICATION

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1 Scope and Applicability

This operating procedure defines the protocol for the preparation of mosquito egg samples for their identification using the MALDI-TOF mass spectrometry technique.

The procedure is applicable to all personnel of the LMA and GLZ involved in the ZT monitoring system.

2 Abbreviations

AF10%	Formic acid 10%
MALDI-TOF MS Spectrometry	Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry
GLZ	Gruppo Lavoro Zanzare
GO-GLZ	Gruppo operativo GLZ
IDTIPIZZ-SM	Area Identificazioni e tipizzazioni – Spettrometria di massa
LMA	Laboratorio Microbiologia Applicata
RH	Relative humidity
RT	Responsabile tecnico
SA	Sinapinic Acid
VET	Area Vettori
ZT	Tiger mosquito (<i>Aedes albopictus</i>)

3 Responsibilities

LMA and GO-GLZ collaborators are responsible for the correct carrying out of the methodology described. RT IDTIPIZZ-SM is responsible for checking that the procedure is properly performed.

4 Equipment and Supplies

Stereomicroscope (Leica EZ40)

Tweezers

Scalpel (Ref. 4060, Clamed)

Needles (Ref. 86.1568.050, Sarstedt)

Eppendorf tubes 0.2ml (Ref. 72.737.002, Sarstedt)

Pipettor P-2 and P-20

Tips 10 µl (Ref. T-300-STK, Brunschwig)

Tips 20 µl (Ref. 70.760.502, Sarstedt)

SA matrix (40 mg sinapinic acid; 600 µl acetonitrile; 400 µl milliQ water; 3 µl concentrated TFA)

Formic acid 10% (v/v in water)

5 Procedure

The preparation of the samples and the MALDI-TOF MS analysis is performed at the LMA.

5.1 Selection of eggs

IMPORTANT NOTE: Only viable eggs (i.e. not dehydrated nor empty shells) are considered for the analysis. Do not pick up the totality of viable eggs! Always leave viable eggs on the paddle for eventually further analysis.

- Carefully remove all cling film from the paddle using the scalpel.
- Divide the whole wooden paddle into sectors of 4 cm with a pen (for a total of 10 sectors: 5 per side, include to each sector, the edge situated in the inferior side from the sector). Sectors are labelled with a “S” followed by a progressive number (S1 to S10) (Figure 1);
- For each sector, pick-up a maximum of 5 single-laid eggs for the analysis. Follow point 5.2 for the preparation of the MALDI-TOF MS target plate (*Single eggs*);
- In case of presence of egg batches (groups of eggs, Figure 2), label each batch with a letter “B” followed by a progressive number (e.g. B1, B2, B3) (Figure 1).
 - If only one batch is present on the paddle, pick-up individually the half of the eggs from the batch, for a maximum of 5 eggs. Follow point 5.2 for the preparation of the MALDI-TOF MS target plate (*Single eggs*).
 - If more than 1 eggs’ batch is present on the paddle, prepare egg pools. For each pool, pick-up a maximum of 5 eggs per batch from a maximum of 3 different batches. Record the number of eggs picked-up from the batches. Follow point 5.2 for the preparation of the MALDI-TOF MS target plate (*Eggs pool*).

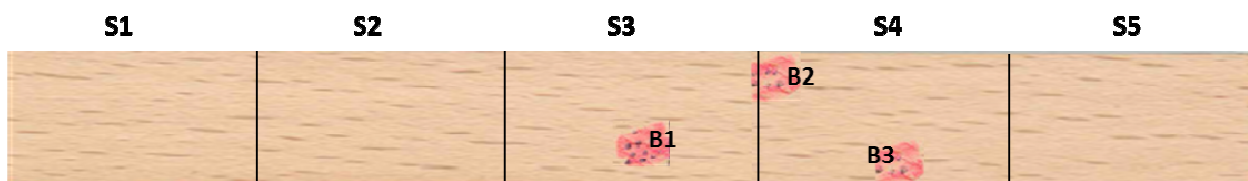


Figure 1. Representation of one side of a wooden paddle. The paddle is divided in sectors numbered with the letter “S”. Eggs batches (represented in red) are numbered with the letter “B”.



Figure 2. Eggs’ batch.

- Identify the samples using a code indicating the location and the name of the ovitrap, the round check (labelled with a “G”), the number of the sector on the paddle (for single-laid eggs), as well as the batch number (for egg batches). Add the acronym “CH” to the code if the eggs originate from ovitraps of the Swiss monitoring system, or TI if the eggs originate from the monitoring in Ticino. Use serial numbers for all the samples originating from a same paddle.

→ TRAP ID_ TI/CH_ROUND CHECK_SECTOR/BATCH_EGG No.

E.g.:

GEN01a-TI-G3-S2-1 (trap GEN-01a of the TI monitoring, round check number 3, sector 2, egg no.1)

GEN01a-TI-G3-B1-1 (trap GEN-01a of the TI monitoring, round check number 3, batch 1, egg no.1)

GEN01a-TI-G3-B1-2 (trap GEN-01a of the TI monitoring, round check number 3, batch 1, egg no.2)

GEN01a-TI-G3-B1-3(9x) (trap GEN-01a of the TI monitoring, round check number 3, eggs from batches 1, 2 and 3, pool of 9 eggs)

AUT11f-CH-G3-S4-1 (trap AUT11f of the Swiss monitoring, round check number 3, sector 4, egg no.1)

- Wrap again the paddle together with its label with the cling film and store it at room temperature in a plastic box.

5.2 Sample preparation for the MALDI-TOF MS analysis

The schema for the transfer of egg samples on the stainless steel target plate (FlexiMass™ (Shimadzu)) is shown in Figure 3.

A1	A2	A3	A4	
B1	B2	B3	B4	
C1	C2	C3	C4	Egg samples
D1	D2	D3	D4	
E1	E2	E3	E4	
F1	F2	F3	F4	
G1	G2	G3	G4	<i>E. coli</i> for calibration
H1	H2	H3	H4	
I1	I2	I3	I4	
J1	J2	J3	J4	Egg samples
K1	K2	K3	K4	
L1	L2	L3	L4	

Figure 3. Schema for the transfer of samples on the FlexiMass™ plate for MALDI-TOF MS.

On positions G3+G4 of each target plate, spot the *E. coli* strain ATCC 8739 (grown overnight at 37°C on blood agar) for the automatic calibration of the instrument as follows:

- Transfer a small quantity of bacteria on the target plate by the means of an orange needle or white loop.
- Add 1µl SA matrix (see SOP [IO MATRICE SA PER MALDI-TOF MS](#)) and let it crystallize at room temperature.

Single eggs:

Spot the eggs individually on the FlexiMass™ target plate as follow:

- With the help of a scalpel and tweezers, add one egg on the spot of the target plate containing 1µl AF10%.
- Crush the egg with the scalpel and tweezers. A yellowish-white material should come out.
- Let dry at room temperature
- Add 1µl SA matrix (see SOP [IO MATRICE SA PER MALDI-TOF MS](#)) and let it crystallize at room temperature.

Eggs pool:

- Pick up a maximum of 5 eggs from each egg batches (for a maximum of 3 different batches) and combine them in pools of a maximum of 15 eggs in a 0.2 ml eppendorf tube.
IMPORTANT NOTE: the number of eggs from each batch has to be identical.
- For every egg, add 1µl AF10% to the eppendorf tube (i.e. 15µl AF10% for a pool of 15 eggs).
- Crush the eggs with an orange needle. Check under the binocular that all the eggs are crushed.
- Add 1µl of the AF10%-eggs suspension on 4 spots of the stainless steel target plate.
- Let the spots dry at room temperature.
- Add 1µl SA matrix (see SOP [IO MATRICE SA PER MALDI-TOF MS](#)) and let it crystallize at room temperature.

Fill in properly the form [MO SCHEMA LAME MALDI-TOF MS](#) and the Excel sheet [MO PROTOCOLLO PIASTRE](#) (add the acronym SUPSI_ before the name of the sample!). The comment file of the [PROTOCOLLO PIASTRE](#) is saved as a text file (.txt) (Testo con valori delimitati da tabulazioni).

5.3 MALDI-TOF MS analysis

Analyse the samples according to the [QUICKGUIDE_ANALISI MALDI-TOF MS ZANZARE](#).

Send the ASCII files by e-mail to MABRITEC AG for identifications (dominik.ziegler@mabritec.com).

6 Regulatory references

None.

7 Literature

Schaffner F., Kaufmann C., Pflüger V., and Mathis A. 2014. Rapid protein profiling facilitates surveillance of invasive mosquito species. *Parasites & Vectors*, 7:142.

9.2 ANNEX 2. Ricerca di nuove specie di zanzare in Ticino mediante analisi MALDI-TOF MS.

*Gruppo cantonale Lotta alle Zanzare (GLZ),
via al Castello, 6952 Canobbio – Ticino*

*Fondazione Bolle di Magadino,
6573 Magadino*

**Ricerca di nuove specie di zanzare in Ticino mediante
analisi MALDI-TOF MS**

Verifica della presenza di nuove zanzare esotiche sul territorio cantonale tramite il controllo a campione delle uova raccolte con la rete di monitoraggio per la Zanzara tigre

Rapporto 2013-2014

Committenti :

Dipartimento della sanità e socialità - Bellinzona. Cantone Ticino.

**Ufficio federale dell'ambiente UFAM – Berna.
(Per parte delle analisi 2014: contratto 00.0303. PZ / N492-2449)**

22 Gennaio 2015

1. Introduzione

A causa dei cambiamenti climatici in atto e della globalizzazione del commercio in Europa stanno arrivando in Svizzera specie esotiche di zanzare, alcune a carattere invasivo, come p.es. la zanzara tigre (*Aedes albopictus*), che possono cambiare lo scenario epidemiologico del continente (Medlock et al. 2012). Infatti, alcune di queste specie sono vettrici di malattie per ora prevalentemente confinate in paesi tropicali, come la febbre gialla, la dengue, la febbre chikungunya, etc.

In Europa c'è stata un'epidemia di chikungunya nel 2007 legata alla presenza di zanzara tigre (Angelini et al. 2007). Inoltre in Francia, sempre legati alla presenza di zanzara tigre, ci sono stati casi di trasmissione locale di chikungunya nel 2010 (Granddam et al. 2011) e nel 2014 (WHO 2014). Nel 2010 e nel 2013 in Francia (La Ruche et al. 2010; Marchand et al. 2013) e in Croazia nel 2010 (Schmidt-Chanasit et al. 2010; Gjenero-Margan et al. 2011) ci sono stati casi locali di dengue. Inoltre nella provincia portoghese di Madeira nel 2012 c'è stata un'epidemia di dengue, con mille casi confermati, legata alla presenza di un'altra specie esotica, l'*Aedes aegypti* (ECDC 2013).

Le specie esotiche ritrovate recentemente in Europa sono: *Ae. aegypti* (Madeira nel 2005), *Ae. albopictus* (Albania nel 1979), *Ae. atropalpus* (Italia nel 1996), *Ae. japonicus* (Francia nel 2000), *Ae. koreicus* (Italia 2011) e *Ae. triseriatus* (Francia nel 2004).

Tutte le specie esotiche sopracitate appartengono al genere *Aedes* e sono considerate "container-breeding mosquitoes", cioè zanzare che si possono riprodurre in contenitori artificiali. Questi luoghi di sviluppo sono prevalentemente rinvenibili in ambiente urbano.

Le trappole usate nel monitoraggio ticinese per la zanzara tigre sono delle ovitrappole, cioè dei contenitori artificiali messi apposta per raccogliere uova di questa specie e rilevarne così la presenza. Su tali trappole possono anche deporre le altre specie invasive esotiche.

Larve o adulti di tali specie possono essere distinti morfologicamente da specialisti, mentre la differenziazione morfologica al binoculare delle uova è praticamente impossibile. In tal caso si usano tecniche biomolecolari o la microscopia elettronica. Queste tecniche sono però molto dispendiose. L'utilizzo della tecnica MALDI-TOF MS (Matrix Assisted Laser desorption/ionization time-of-flight mass spectrometry) permette invece di identificare, in tempi rapidi, le uova delle differenti specie di zanzara con precisione (Schaffner et al. 2014,) e con costi ridotti rispetto alle altre tecniche.

La tecnica MALDI-TOF MS è stata applicata per la prima volta nel monitoraggio sulla zanzara tigre in Ticino nel 2013 per confermare la distinzione visiva/morfologica tra le uova di zanzara tigre e un'altra specie locale, l'*Ae. geniculatus*.

Nello stesso anno, vista l'espansione di *Ae. japonicus* a nord delle Alpi e la possibilità di applicare questa nuova tecnica al monitoraggio, alcuni campioni di uova presunte di zanzara tigre sono stati analizzati con la tecnica MALDI-TOF MS al fine di trovare *Ae. japonicus* o un'altra specie esotica.

2. Materiale e Metodi

Per verificare la presenza o l'assenza di zanzara tigre nel monitoraggio condotto nel Canton Ticino, in oltre 60 comuni, vengono usate trappole per la deposizione di uova, chiamate ovitrappole. In queste trappole, oltre alla zanzara, tigre possono deporre le loro uova anche altre specie appartenenti al genere *Aedes*, tra cui le specie esotiche sopracitate.

Per rilevare la presenza di zanzara tigre in Canton Ticino il sistema di monitoraggio utilizza principalmente delle trappole che rilevano uova deposte da questa specie: le ovitrappole. Le ovitrappole sono costituite da un barattolo di plastica nero della capienza di 1,5 litri che viene riempito con acqua del rubinetto. Nell'acqua viene messo del larvicida biologico per evitare lo sviluppo larvale e un legnetto sul quale le zanzare possono deporre le loro uova. I legnetti vengono raccolti ogni 15 giorni, avvolti in una pellicola di plastica e portati in laboratorio. Qui mediante uno stereoscopio si verifica morfologicamente la presenza o meno di uova di zanzara.

Le uova di specie invasive sono morfologicamente simili tra loro. Per verificare la presenza di specie invasive ulteriori oltre la zanzara tigre, sono state scelte trappole positive in modo casuale da ogni comune facente parte delle rete di monitoraggio. Si è cercato di analizzare con la tecnica MALDI-TOF MS almeno 2 trappole per ogni comune durante la stagione di monitoraggio 2013. Mentre per la stagione 2014, la scelta delle trappole analizzate con questa tecnica si è basata sui risultati ottenuti nel 2013, cioè concentrandosi sulle aree dove si erano trovate specie invasive l'anno precedente.

Per la preparazione dei campioni da trappole selezionate per l'analisi si è proceduto secondo i protocolli allegati (allegato 1 e allegato 2). Unica modifica apportata riguarda la divisione in settori dei legnetti contenenti le uova, che non è stata effettuata. Si è invece scelto di analizzare un massimo di 6 uova per legnetto, se singole, o pools di massimo 20 uova, se presenti uova raggruppate.

3. Risultati

In questo rapporto non sono indicati i campioni da cui si è ottenuto uno spettro di cattiva qualità mediante l'analisi MALDI-TOF MS e che quindi non hanno permesso un'identificazione specifica del campione.

3.1 Risultati stagione 2013

Nel 2013 sono state analizzate con successo complessivamente 3560 uova, tra cui 370 pools (di un numero di uova variabile fra 7 e 20) e 427 uova singole. Tali uova provenivano da 202 ovitrappole diverse, appartenenti al sistema di monitoraggio di 48 comuni differenti e da quello lungo l'asse autostradale ticinese dell'A2.

Complessivamente 22 uova risultate essere *Ae. koreicus* sono state trovate in un'unica trappola nel comune di Breggia (19-23 agosto). In una trappola di Giubiasco è stato trovato un uovo di *Ae. cretinus* (24-28 giugno) (Tab 1).

3.2 Risultati stagione 2014 fino al 14 agosto

Sulla base dei risultati ottenuti nel 2013 si è deciso di effettuare analisi più mirate durante la stagione di monitoraggio 2014. In particolare si è scelto di analizzare uova provenienti dai luoghi in cui nell'anno precedente si erano trovate le specie potenzialmente invasive *Ae. koreicus* e *Ae. cretinus*.

Nel 2013 un lavoro di dottorato patrocinato dall'Istituto tropicale svizzero di Basilea effettuato in collaborazione con il GLZ ha permesso di mettere in evidenza la presenza della specie *Ae. koreicus* (Suter et al. 2014 articolo inoltrato) a Chiasso e in alcuni comuni italiani confinanti. Si è quindi deciso di analizzare trappole provenienti da tutti i comuni di questa fascia di confine. I comuni analizzati per *Ae. koreicus* sono stati: Breggia, Chiasso, Balerna, Coldrerio, Stabio, Vacallo ed Arzo.

Nel comune di Arzo non sono state trovate trappole positive nel 2014, quindi non ci sono analisi. Per gli altri comuni tutti i legnetti con uova in buono stato, provenienti da 68 trappole differenti, sono stati analizzati con la tecnica MALDI-TOF MS.

Nel 2014 ci si è focalizzati anche sulle trappole positive del comune di Giubiasco, visto che l'anno precedente era stata trovata la specie *Ae. cretinus*. Inoltre, grazie alla segnalazione di un cittadino, un adulto di *Ae. japonicus* è stata trovata nel comune di Gambarogno. Attorno al luogo di segnalazione è stato aumentato il numero di ovitrappole, tutte le trappole positive rinvenute sono state in seguito analizzate con la tecnica MALDI-TOF MS.

In totale sono state analizzate 584 uova provenienti da 77 trappole del monitoraggio (su un totale di 541 trappole positive controllate provenienti da questi comuni) provenienti dai comuni di Breggia, Chiasso, Balerna, Coldrerio, Stabio, Vacallo, Giubiasco e Gambarogno, e dal monitoraggio dell'asse autostradale della A2.

Due uova appartenenti alla specie *Ae. cretinus* sono state trovate nel comune di Balerna, in due diverse trappole, su 15 analizzate; mentre 2 uova appartenenti alla specie *Ae. japonicus* sono state trovate nei comuni di Gambarogno (in una trappola su due analizzate) e Giubiasco (in 1 trappola su 10 analizzate) e 18 uova appartenenti alla specie *Ae. koreicus* sono state trovate nei comuni di Coldrerio (15 uova in 1 trappola su 12 analizzate) e Chiasso (3 uova in 1 trappola su 12 analizzate).

Nella tabella 1 e nella figura 1 sono riportati i dati riguardanti le nuove specie invasive trovate nelle varie località nei due anni in esame.

3.3 Risultati stagione 2014 dal 15 agosto al 19 settembre

Durante il mese di dicembre del 2014 grazie alla sovvenzione (Auftrags- Nr. 00.0303. PZ / N492-2449) ricevuta dal Ufficio Federale per l'Ambiente, si sono potute completare le analisi MALDI-TOF MS per la ricerca di nuove specie esotiche della stagione 2014. Complessivamente sono state analizzate 296 uova singole, provenienti da 70 trappole diverse dei comuni di Breggia, Chiasso, Balerna, Coldrerio, Stabio, Vacallo, Giubiasco e Gambarogno (allegato 3).

Sei uova appartenenti alla specie *Ae. japonicus* sono state trovate nel comune di Gambarogno (in 1 trappola su 4 analizzate); 4 uova appartenenti alla specie *Ae. koreicus* sono state trovate nel comune di Chiasso (in 1 trappola su 17 analizzate).

Inoltre 208 uova erano di *Ae. albopictus* e 3 di *Ae. geniculatus*, specie di cui è nota la presenza sul territorio. Le rimanenti 75 uova analizzate non hanno fornito uno spettro di qualità sufficiente all'identificazione. La causa di un numero tanto elevato di non identificazioni è probabilmente attribuibile all'età delle uova. Molte uova infatti sono risultate al momento dell'analisi non in ottimo stato di conservazione.

I risultati ottenuti da queste ultime analisi hanno permesso di confermare la presenza di *Ae. koreicus* nel comune di confine, infatti questa specie è stata trovata nel 2014 a Chiasso sia a fine luglio che a metà settembre e di *Ae. japonicus* nel Gambarogno, dove è stata trovata sia in agosto che a settembre. Non si è invece confermata la presenza di *Ae. cretinus* (vedi Tabella 1)

Anno	identificazione MALDI-TOF	Comune	Raccolta uova	N° uova	LV03_Est	LV03_Nord	Trappola	Analisi uova
2013	<i>Aedes cretinus</i>	Giubiasco	22.07-26.07	1	721071	115229	GIU_06a	singole
2013	<i>Aedes koreicus</i>	Breggia	19.08-23.08	2	723170	79484	BRE_03b	singole
2013	<i>Aedes koreicus</i>	Breggia	19.08-23.08	20	723170	79484	BRE_03b	pool
2014	<i>Aedes cretinus</i>	Balerna	07.07-11.07	1	721563	79186	BAL_01a	singole
2014	<i>Aedes cretinus</i>	Balerna	07.07-11.07	1	721647	78388	BAL_08b	singole
2014	<i>Aedes koreicus</i>	Coldrerio	23.06-27.06	15	720355	78869	COL_15b	pool
2014	<i>Aedes koreicus</i>	Chiasso	21.07-25.07	3	723353	77504	CHI_03b	singole
2014	<i>Aedes koreicus</i>	Chiasso	15.09-19.09	4	722391	76183	CHI_22b	singole
2014	<i>Aedes japonicus</i>	Giubiasco	09.06-13.06	1	722005	115311	GIU_05a	singole
2014	<i>Aedes japonicus</i>	Gambarogno	04.08-08.08	1	706979.4	110178	MAG_22a	singole
2014	<i>Aedes japonicus</i>	Gambarogno	01.09-05.09	2	706965.5	110194.2	MAG_22b	singole
2014	<i>Aedes japonicus</i>	Gambarogno	15.09-19.09	4	706975.9	110210.3	MAG_22b	singole

Tabella 1. Identificazione nuove specie esotiche in Ticino 2013-2014

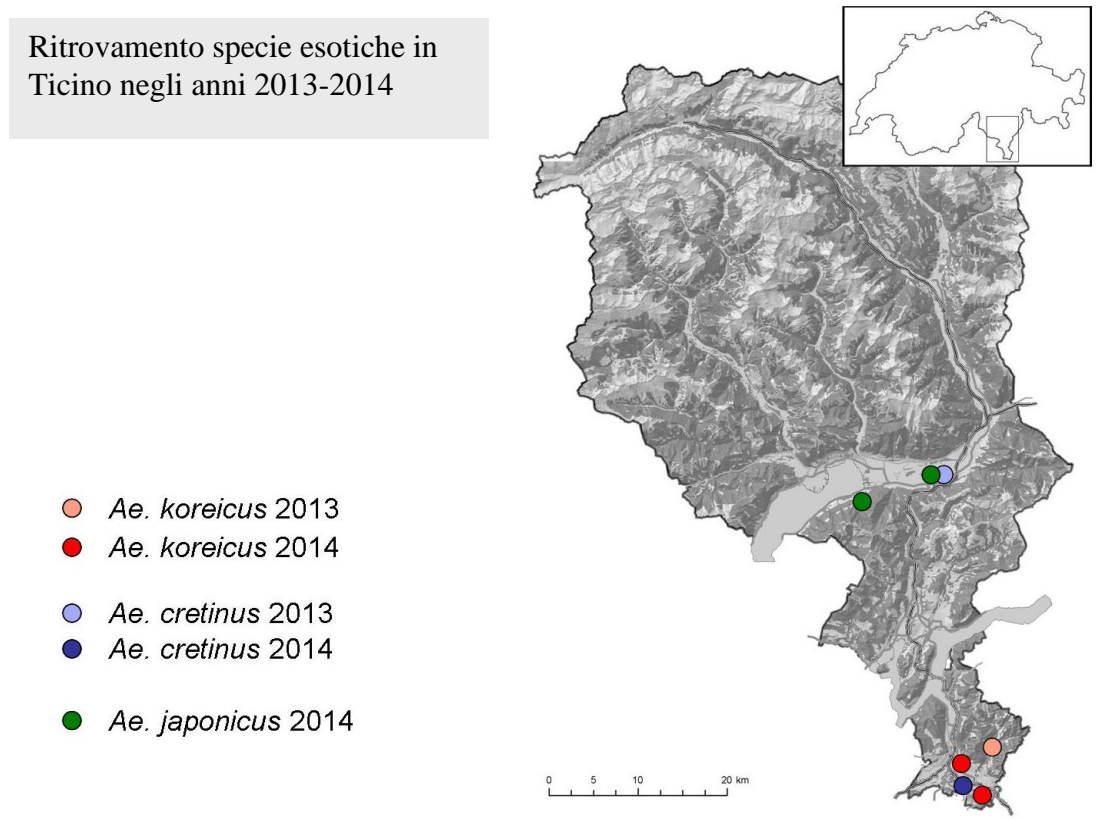


Figura 1. Localizzazione nuove specie esotiche in Ticino

4. Discussione

Segnalata per la prima volta in Europa nel 2000, al Nord della Francia, e successivamente in Belgio, Svizzera e Germania (<http://www.ecdc.europa.eu/en/healthtopics/vectors/mosquitoes/Pages/aedes-japonicus.aspx#geo>), *Aedes japonicus* è presente dal 2008 in Svizzera, a Nord delle Alpi.

In Giappone e Corea, luoghi d'origine di *Ae. japonicus*, la specie non è considerata un importante vettore di malattie (<http://www.ecdc.europa.eu/en/healthtopics/vectors/mosquitoes/Pages/aedes-japonicus.aspx>). Tuttavia recentemente è stata dimostrata in laboratorio la possibile competenza vettoriale di questa specie per la trasmissione di virus per le malattie dengue e chikungunya (Schaffner et al. 2011).

Aedes koreicus al di fuori della sua area di origine, la Corea, è stata rinvenuta in Belgio nel 2008, dove tuttavia non sembra diffondersi (Versteirt et al. 2012). Un ritrovamento si è verificato anche in Italia, nel Veneto nel 2011 (Capelli et al. 2011). In alcune parti della Russia *Ae. koreicus* ha dimostrato essere vettore dell'encefalite giapponese (Miles 1964). Ci sono inoltre indicazioni secondo cui questa specie sarebbe in grado di trasmettere i nematodi *Dirofilaria immitis* al cane e *Brugia malayi* all'uomo (<http://www.ecdc.europa.eu/en/healthtopics/vectors/mosquitoes/Pages/aedes-koreicus.aspx>).

Per quanto riguarda *Ae. cretinus*, si è a conoscenza di una sua diffusa presenza solamente in Grecia, Macedonia, Georgia e in Turchia (Lane et al. 1982). Di *Ae. cretinus* non si sa molto, né come habitat né come capacità vettoriale. Questa specie è molto simile alla zanzara tigre e può ibridarsi con essa. Studi a riguardo sono ancora in corso.

Tutte queste specie sono "container breeding mosquitoes", quindi amano riprodursi in piccole raccolte d'acqua che possono trovare sia in ambito urbano (recipienti vari, tombini, ecc), che in ambito naturale (cavità di alberi).

5. Conclusione

Le analisi effettuate nei due anni passati ci hanno permesso di identificare tre nuove specie esotiche di *Aedes* in Ticino.

La presenza di *Ae. koreicus* nella regione del Mendrisiotto è confermata.

Le analisi MALDI-TOF MS hanno permesso di confermare anche la presenza di *Ae. japonicus* nel Gambarogno .

Un adulto. *Ae. japonicus* è stato inoltre rilevato anche nel Bellinzonese, ma non possiamo ancora dire se siamo confrontati con una piccola popolazione stabile in zona.

La presenza di *Ae. cretinus* andrebbe invece ulteriormente confermata, possibilmente con campagne di catture di adulti mirate e con ulteriori pose di ovitrappole. Le uova identificate sono infatti finora troppo poche per sostenere che la specie sia presente in modo stabile sul territorio cantonale.

La capacità vettoriale di queste tre specie esotiche rinvenute non è molto chiara, studi al riguardo sono tuttora in corso. *Ae. japonicus* ha dimostrato di riprodursi bene in condizioni climatiche presenti a nord delle Alpi. *Ae. koreicus* sembra aver trovato ambienti riproduttivi adatti a quote superiori dove normalmente viene trovata la zanzara tigre (Dr. Drago e Dr. Capelli comunicazioni personali). A nostro parere queste specie dovrebbero essere monitorate in quanto potrebbero avere un effetto sinergico con la zanzara tigre nel rischio sanitario, esser potrebbero cioè portare malattie esotiche in aree dove la zanzara tigre non riesce a stabilirsi e non entra in competizione per i siti di riproduzione.

In futuro è dunque consigliabile poter proseguire la ricerca di tali specie esotiche nel territorio utilizzando sia i metodo classici d'indagine, sia la tecnica MALDI-TOF MS applicata per l'analisi delle uova provenienti dalle ovitrappole e per monitoraggio della zanzara tigre.

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Analisi MALDI-TOF effettuate grazie a contratto BAFU Nr. 00.0303. PZ / N492-2449

identificazione MALDI-TOF	Trappola	LV03_Est	LV03_Nord	Comune	Raccolta uova	analisi uova	N° uova
<i>Aedes albopictus</i>	BAL-11a	721275	78175	Balerna	18.08-22.08	singole	1
<i>Aedes albopictus</i>	BAL-01a	721563	79186	Balerna	18.08-22.08	singole	1
<i>Aedes albopictus</i>	BAL-02a	721738	78128	Balerna	15.09-19.09	singole	3
<i>Aedes albopictus</i>	BAL-21b	721793	77338	Balerna	15.09-19.09	singole	3
<i>Aedes albopictus</i>	BAL-19a	722738	77758	Balerna	15.09-19.09	singole	3
<i>Aedes albopictus</i>	BAL-08b	721647	78388	Balerna	15.09-19.09	singole	4
<i>Aedes albopictus</i>	BAL-07a	721022	78518	Balerna	15.09-19.09	singole	2
<i>Aedes albopictus</i>	BAL-11a	721275	78175	Balerna	15.09-19.09	singole	1
<i>Aedes albopictus</i>	BAL-12b	721898	78118	Balerna	15.09-19.09	singole	2
<i>Aedes albopictus</i>	BAL-04a	721298	78624	Balerna	15.09-19.09	singole	3
<i>Aedes japonicus</i>	MAG-22b	706965.5	110194.2	Gambarogno	15.09-19.09	singole	4
<i>Aedes albopictus</i>	GIU-15a	721836	114790	Giubiasco	15.09-19.09	singole	3
<i>Aedes albopictus</i>	GIU-07b	721067	114639	Giubiasco	15.09-19.09	singole	1
<i>Aedes albopictus</i>	GIU-06b	720723	114887	Giubiasco	15.09-19.09	singole	3
<i>Aedes albopictus</i>	GIU-14a	721428	114382	Giubiasco	15.09-19.09	singole	3
<i>Aedes albopictus</i>	GIU-10b	720591	114486	Giubiasco	15.09-19.09	singole	2
<i>Aedes albopictus</i>	GIU-05a	722005	115311	Giubiasco	15.09-19.09	singole	4
<i>Aedes albopictus</i>	STA-03b	716430	79657	Stabio	15.09-19.09	singole	3
<i>Aedes albopictus</i>	STA-01b	716098	79742	Stabio	15.09-19.09	singole	2
<i>Aedes albopictus</i>	STA-17a	716430	79119	Stabio	15.09-19.09	singole	1
<i>Aedes albopictus</i>	STA-09b	716395	78869	Stabio	15.09-19.09	singole	2
<i>Aedes albopictus</i>	STA-05b	716715	79432	Stabio	15.09-19.09	singole	3
<i>Aedes albopictus</i>	STA-21b	715696	78080	Stabio	15.09-19.09	singole	1
<i>Aedes albopictus</i>	STA-06b	716373	79142	Stabio	15.09-19.09	singole	1
<i>Aedes albopictus</i>	STA-19b	716861	78329	Stabio	15.09-19.09	singole	2
<i>Aedes albopictus</i>	STA-23a	715175	77783	Stabio	15.09-19.09	singole	4
<i>Aedes albopictus</i>	CHI-21b	721146	76598	Chiasso	15.09-19.09	singole	3
<i>Aedes albopictus</i>	CHI-11a	722746	77165	Chiasso	15.09-19.09	singole	3
<i>Aedes albopictus</i>	CHI-20b	723964	76905	Chiasso	15.09-19.09	singole	1
<i>Aedes koreicus</i>	CHI-22b	722391	76183	Chiasso	15.09-19.09	singole	4
<i>Aedes albopictus</i>	CHI-04a	723701	77543	Chiasso	15.09-19.09	singole	3
<i>Aedes albopictus</i>	CHI-04b	723919	77519	Chiasso	15.09-19.09	singole	2
<i>Aedes albopictus</i>	CHI-18a	723053	76913	Chiasso	15.09-19.09	singole	4
<i>Aedes albopictus</i>	CHI-21a	721481	76273	Chiasso	15.09-19.09	singole	2
<i>Aedes albopictus</i>	CHI-16a	724144	77050	Chiasso	15.09-19.09	singole	2
<i>Aedes albopictus</i>	CHI-06a	723431	77290	Chiasso	15.09-19.09	singole	3
<i>Aedes albopictus</i>	CHI-06b	723442	77431	Chiasso	15.09-19.09	singole	3
<i>Aedes albopictus</i>	CHI-13b	723463	77153	Chiasso	15.09-19.09	singole	2
<i>Aedes albopictus</i>	GIU-09a	720519	114711	Giubiasco	18.08-22.08	singole	1
<i>Aedes albopictus</i>	COL-14b	720170	78912	Coldrerio	18.08-22.08	singole	2
<i>Aedes albopictus</i>	COL-10b	720152	79029	Coldrerio	18.08-22.08	singole	3
<i>Aedes albopictus</i>	COL-12a	720639	78985	Coldrerio	01.09-05.09	singole	2
<i>Aedes geniculatus</i>	COL-12a	720639	78985	Coldrerio	01.09-05.09	singole	1
<i>Aedes albopictus</i>	COL-10b	720152	79029	Coldrerio	01.09-05.09	singole	3
<i>Aedes albopictus</i>	COL-06a	720108	79469	Coldrerio	01.09-05.09	singole	2
<i>Aedes albopictus</i>	COL-09b	720794	79359	Coldrerio	01.09-05.09	singole	3
<i>Aedes albopictus</i>	COL-09b	720794	79359	Coldrerio	15.09-19.09	singole	1
<i>Aedes albopictus</i>	COL-14b	720170	78912	Coldrerio	15.09-19.09	singole	2
<i>Aedes albopictus</i>	COL-10b	720152	79029	Coldrerio	15.09-19.09	singole	3
<i>Aedes albopictus</i>	BRE-08a	724404	79592	Breggia	01.09-05.09	singole	1
<i>Aedes geniculatus</i>	BRE-05a	723424	80165	Breggia	01.09-05.09	singole	2

<i>Aedes albopictus</i>	BRE-02b	722943	79723	Breggia	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-17a	723444	77684	Vacallo	15.09-19.09	singole	3
<i>Aedes albopictus</i>	VAC-01a	723825	79003	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-13a	724144	78046	Vacallo	15.09-19.09	singole	5
<i>Aedes albopictus</i>	VAC-14a	723277	77800	Vacallo	15.09-19.09	singole	3
<i>Aedes albopictus</i>	VAC-08a	723706	78365	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-11a	723609	78075	Vacallo	15.09-19.09	singole	3
<i>Aedes albopictus</i>	VAC-11b	723569	78147	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-17b	723305	77725	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-17b	723305	77725	Vacallo	15.09-19.09	singole	3
<i>Aedes albopictus</i>	VAC-07a	724730	78595	Vacallo	15.09-19.09	singole	3
<i>Aedes albopictus</i>	VAC-12b	723925	78027	Vacallo	15.09-19.09	singole	3
<i>Aedes albopictus</i>	VAC-04b	723951	78564	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-04a	723944	78605	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	VAC-08b	723639	78367	Vacallo	15.09-19.09	singole	2
<i>Aedes albopictus</i>	STA-04b	716758	79509	Stabio	18.08-22.08	singole	1
<i>Aedes albopictus</i>	STA-05b	716715	79432	Stabio	18.08-22.08	singole	1
<i>Aedes albopictus</i>	STA-14a	716901	78462	Stabio	01.09-05.09	singole	1
<i>Aedes albopictus</i>	STA-07a	716530	79034	Stabio	01.09-05.09	singole	2
<i>Aedes albopictus</i>	STA-18b	715556	77777	Stabio	01.09-05.09	singole	2
<i>Aedes albopictus</i>	STA-03b	716430	79657	Stabio	01.09-05.09	singole	3
<i>Aedes albopictus</i>	VAC-14a	723277	77800	Vacallo	01.09-05.09	singole	1
<i>Aedes albopictus</i>	VAC-17a	723444	77684	Vacallo	01.09-05.09	singole	1
<i>Aedes albopictus</i>	VAC-12a	723852	78081	Vacallo	01.09-05.09	singole	1
<i>Aedes albopictus</i>	VAC-11a	723609	78075	Vacallo	01.09-05.09	singole	1
<i>Aedes albopictus</i>	VAC-01a	723825	79003	Vacallo	01.09-05.09	singole	1
<i>Aedes japonicus</i>	MAG-22b	706965.5	110194.2	Gambarogno	01.09-05.09	singole	2
<i>Aedes albopictus</i>	MAG-22c	706975.9	110210.3	Gambarogno	01.09-05.09	singole	1
<i>Aedes albopictus</i>	BAL-04a	721298	78624	Balerna	01.09-05.09	singole	2
<i>Aedes albopictus</i>	BAL-19a	722738	77758	Balerna	01.09-05.09	singole	1
<i>Aedes albopictus</i>	BAL-21b	721793	77338	Balerna	01.09-05.09	singole	3
<i>Aedes albopictus</i>	BAL-13b	722093	77975	Balerna	01.09-05.09	singole	1
<i>Aedes albopictus</i>	BAL-09a	721885	78233	Balerna	01.09-05.09	singole	2
<i>Aedes albopictus</i>	BAL-08b	721647	78388	Balerna	01.09-05.09	singole	3
<i>Aedes albopictus</i>	BAL-11a	721275	78175	Balerna	01.09-05.09	singole	2
<i>Aedes albopictus</i>	BAL-12a	721738	78128	Balerna	01.09-05.09	singole	3
<i>Aedes albopictus</i>	BAL-02b	721654	79008	Balerna	01.09-05.09	singole	1
<i>Aedes albopictus</i>	BAL-23a	722301	77175	Balerna	01.09-05.09	singole	2
<i>Aedes albopictus</i>	CHI-06b	723442	77431	Chiasso	01.09-05.09	singole	1
<i>Aedes albopictus</i>	CHI-18a	723053	76913	Chiasso	01.09-05.09	singole	1
<i>Aedes albopictus</i>	CHI-21b	721146	76598	Chiasso	01.09-05.09	singole	1
<i>Aedes albopictus</i>	STA-23a	715175	77783	Stabio	01.09-05.09	singole	1
<i>Aedes albopictus</i>	STA-17a	716430	79119	Stabio	01.09-05.09	singole	2
<i>Aedes albopictus</i>	STA-01b	716098	79742	Stabio	01.09-05.09	singole	1
<i>Aedes albopictus</i>	BAL-23a	722301	77175	Balerna	01.09-05.09	singole	1
<i>Aedes albopictus</i>	CHI-06b	723442	77431	Chiasso	01.09-05.09	singole	1
<i>Aedes albopictus</i>	CHI-18a	723053	76913	Chiasso	01.09-05.09	singole	1
<i>Aedes albopictus</i>	CHI-21b	721146	76598	Chiasso	01.09-05.09	singole	1

9.3 ANNEX 3. List of mosquito species reported in Canton Ticino until 01.08.2015 and their vector competence for pathogens (reported in EU) with medical or veterinary interest

Mosquito species reported in Ticino	Literature	Environment (prevalent in this study)	Presence on the territory	Mosquito-borne pathogens (reported in EU) with some impact on human health
<i>Anopheles (Anopheles) claviger</i> (Meigen, 1804)	(Borrani 1937; Briegel 1973; Fouque et al.1991; Galli-Valerio 1905; Vogel 1931; Flacio et al. 2014)	natural	low	BATV, TAHV
<i>An. (Ano.) maculipennis</i> (Meigen, 1818)	(Borrani 1937; Briegel 1973; Focarile 1987; Fouque et al.1991; Galli-Valerio 1905)			Plasmodium sp
<i>An. (Ano.) maculipennis</i> s.l. (Meigen, 1818)	(Flacio et al. 2014)	natural	medium	BATV, Plasmodium sp, USUV, WNV
<i>An. (Ano.) messeae</i> (Falleroni, 1926)	(Briegel et al. 2002)			Plasmodium sp
<i>An. (Ano.) plumbeus</i> (Stephens, 1828)	(Borrani 1937; Briegel 1973; Focarile 1987; Vogel 1931; Flacio et al. 2014)	natural	low	Plasmodium sp, WNV
<i>Aedes (Aedes) cinereus</i> (Meigen, 1818)	(Focarile 1987; Fouque et al.1991)			SINV, TAHV, WNV, (SSHV)
<i>Aedes (Aedes) cinereus / geminus</i>	(Flacio et al. 2014)	natural	low	
<i>Ae. (Aedimorphus) vexans vexans</i> (Meigen, 1830)	(Fouque et al.1991; Fouque et al.1998; Lüthy 1987; Flacio et al. 2014)	natural	high	Nematodes, TAHV, WNV, (SSHV)
<i>Ae. (Finlaya) geniculatus</i> (Olivier, 1791)	(Borrani 1937; Briegel 1973; Focarile 1987; Vogel 1931; Flacio et al. 2014)	natural and urban	medium	CHK, WNV, YFV
<i>Aedes (Fin.) japonicus japonicus</i> (Theobald, 1901)	(GLZ and FBM 2015)	urban	low	CHKV, DENV, WNV (?)
<i>Aedes (Fin.) koreicus</i> (Edwards, 1917)	(GLZ and FBM 2015; Suter et al. 2015)	urban	low	Nematodes
<i>Ae. (Ochlerotatus) annulipes</i> (Meigen, 1830)	(Fouque et al.1991, Flacio et al. 2014)	natural	low	TAHV

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Mosquito species reported in Ticino	Literature	Environment (prevalent in this study)	Presence on the territory	Mosquito-borne pathogens (reported in EU) with some impact on human health
<i>Ae. (Och.) cantans</i> (Meigen, 1818)	(Focarile 1987; Fouque et al.1991; Flacio et al. 2014)	natural	low	TAHV, WNV
<i>Ae. (Och.) caspius caspius</i> (Pallas, 1771)	(Flacio et al. 2014)	natural	low	TAHV, USUV, WNV, Nematodes,
<i>Ae. (Och.) cataphylla</i> (Dyar, 1916)	(Flacio et al. 2014)	mountain	low	(SSHV)
<i>Ae. (Och.) communis</i> (De Geer, 1776)	(Briegel 1973)			TAHV, SINV, BATV, (SSHV), (INKV)
<i>Ae. (Och.) intrudens</i> (Dyar, 1919)	(Fouque et al.1991; Flacio et al. 2014)	natural	low	
<i>Ae. (Och.) pullatus</i> (Coquillett, 1904)	(Borrani 1937)			
<i>Ae. (Och.) punctor</i> (Kirby, 1837)	(Fouque et al.1991)			TAHV, WNV, BATV, (SSHV), (INKV)
<i>Ae. (Och.) sticticus</i> (Meigen, 1838)	(Borrani 1937; Fouque et al.1991; Flacio et al. 2014)	natural	high	TAHV
<i>Ae. (Rus.) rusticus</i> (Rossi, 1790)	(Focarile 1987; Flacio et al. 2014)	natural	low	
<i>Ae. (Stegomyia) albopictus</i> (Skuse, 1894)	(Flacio et al. 2004; Merz et al. 2006)	urban	high	CHKV, DENV, SINV, YFV, USUV (?), WNV
<i>Aedes (Steg.) cretinus</i> (Edwards, 1921)	(GLZ and FBM 2015)	urban	low (?)	DENV (?)
<i>Coquillettidia (Coquillettidia) buxtoni</i> (Edwards, 1923)	(Flacio et al. 2014)	natural	low	
<i>Cq. (Coq.) richiardii</i> (Ficalbi, 1889)	(Flacio et al. 2014)	natural	medium	TAHV, WNV
<i>Culex (Barraudius) modestus</i> (Ficalbi, 1890)	(Flacio et al. 2014)	natural	low	SINV, TAHV, WNV

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Mosquito species reported in Ticino	Literature	Environment (prevalent in this study)	Presence on the territory	Mosquito-borne pathogens (reported in EU) with some impact on human health
<i>Cx. (Culex) pipiens</i> (Linnaeus, 1758)	(Borrani 1937; Briegel 1973; Fouque et al.1991; Lüthy 1987; Vogel 1931)			SINV, TAHV, USUV, WNV
<i>Cx. (Cux.) pipiens / torrentium</i>	(Flacio et al. 2014)	natural and urban	high	
<i>Cx. (Cux.) theileri</i> (Theobald, 1903)	(Fouque et al.1991)			SINV, WNV
<i>Cx. (Cux.) torrentium</i> (Martini, 1925)	(Fouque et al.1991)			SINV
<i>Cx. (Maillotia) hortensis hortensis</i> (Ficalbi, 1890)	(Fouque et al.1991; Vogel 1931; Flacio et al. 2014)	natural	medium	
<i>Cx. (Neoculex) martinii</i> (Medschid, 1930)	(Flacio et al. 2014)	natural	low	
<i>Cx. (Ncx.) territans</i> (Walker, 1856)	(Focarile 1987; Fouque et al.1991; Flacio et al. 2014)	natural	low	
<i>Cs. (Culiseta) annulata</i> (Schrank, 1776)	(Borrani 1937; Fouque et al.1991; Flacio et al. 2014)	natural	medium	TAHV, USUV, WNV
<i>Orthopodomyia pulchripalpis</i> (Rondani, 1872)	(Fouque et al.1991)			

Abbreviations: Batai virus (BATV), Chikungunya virus (CHKV), Dengue virus (DENV)², Inkoo virus (INKV)³, Sindbis virus (SINV), Snowshoe Hare virus (SSHV)⁴, Tahyna virus (TAHV), Usutu virus (USUV)⁵, West Nile virus (WNV)⁶, Yellow fever virus (YFV)

² *Ae. cretinus* is suspected to be a vector of DENV (Schaffner and Mathis 2014).

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Mosquito species reported in Canton Ticino until 2015 are listed in the Table (Galli-Valerio 1905, Vogel 1931, Borrani 1937, Briegel 1973, Fouque et al. 1991, Flacio et al. 2004, 2014, Merz et al. 2006, Gruppo cantonale di Lavoro Zanzare and Fondazione Bolle di Magadino 2015, Suter et al. 2015) and their vector competence for pathogens with some medical or veterinary interest known to circulate in Europe (Jetten and Takken 1994, Hubálek et al. 1996, Hubálek and Halouzka 1999, Lundström 1999, Sardelis and Turell 2001, Anderson et al. 2004, Gratz 2004, Turell et al. 2005, Hubálek 2008b, Schaffner et al. 2009, Calzolari et al. 2010, 2012, Tamba et al. 2010, Engler et al. 2013, Montarsi et al. 2013, Huber et al. 2014, Kampen and Werner 2014, Schaffner and Mathis 2014, Ashraf et al. 2015, Mercier et al. 2015, Wagner et al. 2015, Zielke et al. 2015).

³ Inkoo virus (INKV) is always indicated in brackets, because its distribution is restricted to northern Europe including Russia (Hubálek 2008a), therefore its occurrence at our latitude is considered unlikely.

⁴ Snowshoe Hare virus (SSHV) is always indicated in brackets, because its natural foci are tundra and taiga biomes (Hubálek 2008a).

⁵ *Ae. albopictus* vector capacity for USUV is not excluded (Calzolari et al. 2012).

⁶ In the United States, *Ae. japonicus* is considered vector competent for this virus (Sardelis and Turell 2001, Turell et al. 2001), whereas a study on a *Ae. japonicus* population in South Germany reported that this mosquito is refractory to the virus (Huber et al. 2014). This could be explained by the restricted population analysed (Zielke et al. 2015), in fact a research at the University of Zurich, showed opposite results, that is that *Ae. japonicus* collected in Switzerland is vector competent for WNV (Wagner et al. 2015).

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