Spatio-temporal diversity of the mosquito fauna in Switzerland (2011-2012)

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Spatio-temporal diversity of the mosquito fauna (Diptera: Culicidae) in Switzerland

Research study

Final report

Commissioned by the Swiss Federal Office for the Environment

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Abstract

The spatio-temporal diversity of the Swiss mosquito fauna was investigated at eight locations distributed throughout the country (altitude between 198 and 1,255 meters above sea level) by five samplings over two years (July 2011 to September 2012). At each location at two sites (nature zone, suburban zone), mosquito immature stages were collected in 15-30 larval habitats (LH), and adults were trapped with CO_2-baited traps. Mosquitoes were morphologically identified to species or sister taxa / species complex level. Mosquitoes were found at all sites. Among the 1,362 LHs inspections, 36.7% revealed the presence of mosquito immature stages, and around 9,000 specimens were identified. Adult trapping yielded around 2,000 mosquitoes. Fifteen mosquito species, 3 sibling species (of which all 6 species were confirmed) and the *Anopheles maculipennis* complex were collected. The nature zones showed the higher diversity (all species observed) but a lower relative abundance as compared to the suburban zones (overall 11 species and 2 sibling species identified). The most abundant species was *Culex. pipiens*/*torrentium* (61.2% of all specimens collected) occurring at all except two sites. Six species were frequently observed (*Cx. hortensis*, *Aedes japonicus*, *An. maculipennis* complex members, *Ae. vexans*, *Ae. cinereus*/*geminus*, and *Ae. sticticus*, accounting for 8.5% to 3.9%, respectively, of the individuals). Six of these seven most abundant species have a potential to act as vector. The highest relative abundances of the mosquito fauna, all species, were observed in June and July, for both nature and suburban zones, with peak abundances of different species varying from June to September.

The experimental approach (repeated larval sampling and CO_2-baited trapping) looks reliable for collecting most of the mosquito species. From the 36 mosquito species known to Switzerland, only 13 were not detected during the study: *Ae. albopictus* which is present in southern Ticino outside the study area only; 12 other species which are known to be rare or to occur at high altitudes. In addition to *Ae. albopictus*, the other invasive species *Ae. japonicus* shows a remarkable distribution, being highly abundant in north-eastern Switzerland (being the third most common species overall), but absent from western and southern parts of the country. All other species do not display any particular distribution.

Further work to be accomplished includes the molecular identification within sibling species /complexes (e.g. *Cx. pipiens*/*torrentium*) by MALDI-TOF mass spectrometry, which has recently been established and which can be used as a high-throughput, cost-efficient and reliable tool for the identification of mosquito adults, larvae and eggs. Finally, extrapolation and modelling the potential distribution of the most common species for risk mapping will be done, using the VECMAP system modelling component which currently is in the finalization process.

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Abbreviations

Ae.	*Aedes*
An.	*Anopheles*
Cq.	*Coquillettidia*
Cs.	*Culiseta*
CSCF	Centre Suisse de Cartographie de la Faune
Cx.	*Culex*
ICM	Istituto Cantonale di Microbiologia, Bellinzona
IPZ	Institute of Parasitology, Zürich
LH	Larval habitat
MCZ	Musée Cantonal de Zoologie, Lausanne
Swiss TPH	Swiss Tropical and Public Health Institute, Basel

Introduction

Background

Mosquitoes are the most important insect vectors worldwide, and the epidemiology of many of the infectious agents they transmit is changing, mainly due to environmental changes as well as increase of trade and tourism [1]. In Switzerland, the significance of mosquitoes currently is restricted to their role as nuisance and as vectors of few pathogens of veterinary importance [2, 3], but this picture might change in the future (e.g. with regard to transmission of West Nile virus which is emerging in neighbouring countries [4]). Further, invasive mosquitoes might pose a hazard for biodiversity [5].

In order to gain an overview on the Swiss mosquito fauna, a recent pilot study [6] was done, combining literature analysis with field investigations along transects in five regions in Switzerland (northern Ticino, Midplains, Inner Alps, Pre-Alps, Jura) encompassing urban, peri-urban, rural and nature zones. Hence, the literature revealed the report of 41 mosquito species in Switzerland, two of them being recent invaders (*Aedes albopictus*, *Ae. japonicus*). However, five species might be considered as doubtful records since only single observations have been reported. Thus, the consolidated list of Swiss mosquitoes currently comprises 36 species. Based on the transect study, the highest diversity of the mosquito fauna was observed in the nature zones where all 13 species identified in this field study were present. In contrast, only 3-4 species were occurring in the urban zones. Also, the spread of the invasive species *Ae. japonicus* was further evaluated along other transects, revealing an expansion of 12 to 43 km in different directions in 2010. Finally, the vector potentials of the mosquitoes of Switzerland were assessed based on literature data, revealing that several of the identified species are putative vectors for a number of pathogens (arboviruses and malaria of medical importance; arboviruses, protozoa and nematodes of veterinary importance). By also including a score for a potential threat to biodiversity, an overall classification of the Swiss mosquitoes with regard to the global threat they pose is obtained (Table 1). Further investigations aiming at assessing the hazard risk may focus on species showing a relevant score equal or higher to 3 in one of the categories.

Framework

Here, we present the results of a research study implemented at the request of the Swiss Federal Office for the Environment (FOEN), under Contract Number 10.0002.PJ / K222-2990.

This study aims at investigating the Swiss mosquito fauna by expanding the investigations reported by the above-mentioned pilot study [6], focusing on the spatio-temporal diversity of the mosquito fauna at eight locations distributed throughout the country as determined by repeated sampling over two years.

As a result, we gain an overview on the Swiss mosquito fauna at country scale, which might serve as a baseline for the assessment of risk hazards (for human or animal health, biodiversity) posed by mosquito species.

Table 1. Classification of Swiss mosquito species with regards to potential threat to human health for 9 arboviruses (HHA), to human health for human malaria (HHM), to animal health for several pathogens (avian malaria, 3 filarial nematodes and 3 arboviruses) (AH), and to biodiversity (TB). Scoring for HH and AH is explained in the pilot study report [6], and scoring for TB is based mainly on invasiveness of the species (see Box 1). A score equal or higher to 3 indicates relevance for the category. Species are listed with descending total score which represents the global potential threat. Species for which score = 0 are not listed.

	HHA	HHM	AH	TB	Global threat
Ae. albopictus	5		5	3	**13**
An. claviger s.s.	3	4	5		**12**
Cx. modestus	5		5		**10**
Cx. pipiens	5		5		**10**
Ae. japonicus	3		3	3	**9**
An. maculipennis s.s.	1	3	5		**9**
Ae. vexans	3		5		**8**
An. plumbeus	2	3	2		**7**
Ae. caspius	1		5		**6**
Ae. dorsalis	3		3		**6**
Cs. longiareolata	2		4		**6**
Ae. punctor	3		2		**5**
Cs. annulata	1		4		**5**
Cs. morsitans	4		1		**5**
Cx. theileri	4		1		**5**
Ae. cinereus s.l.	4				**4**
Ae. geniculatus	2		2		**4**
An. messeae	1	3			**4**
Cq. richiardii	1		3		**4**
Cx. torrentium	4				**4**
Ae. communis	3				**3**
Ae. annulipes	1		1		**2**
Ae. cantans	1		1		**2**
Ae. excrucians s.l.	1		1		**2**
Ae. flavescens	2				**2**
Ae. sticticus	1				**1**

Box 1. Scoring for TB is attributed as follows:

5 – Exotic and invasive species, suppressing indigenous species

4 – Exotic and invasive species, displacing indigenous species

3 – Exotic species, invasive

2 – Exotic species, introduced and established

1 – Indigenous species, expanding to new areas or new larval habitats

0 – Indigenous species, no expansion

1. Material and Methods

Research plan

The pilot study investigating urban, suburban, rural and nature areas at different altitudes had demonstrated that the methodology used was suitable to obtain data for a large spectrum of mosquito species, the nature zone showing the highest diversity. Therefore, for the present study, the spectrum of land-use units to be investigated was reduced, by selecting (1) the nature zone for high diversity, and (2) the suburban zone for high mosquito-host (human, pets and livestock) contact rate.

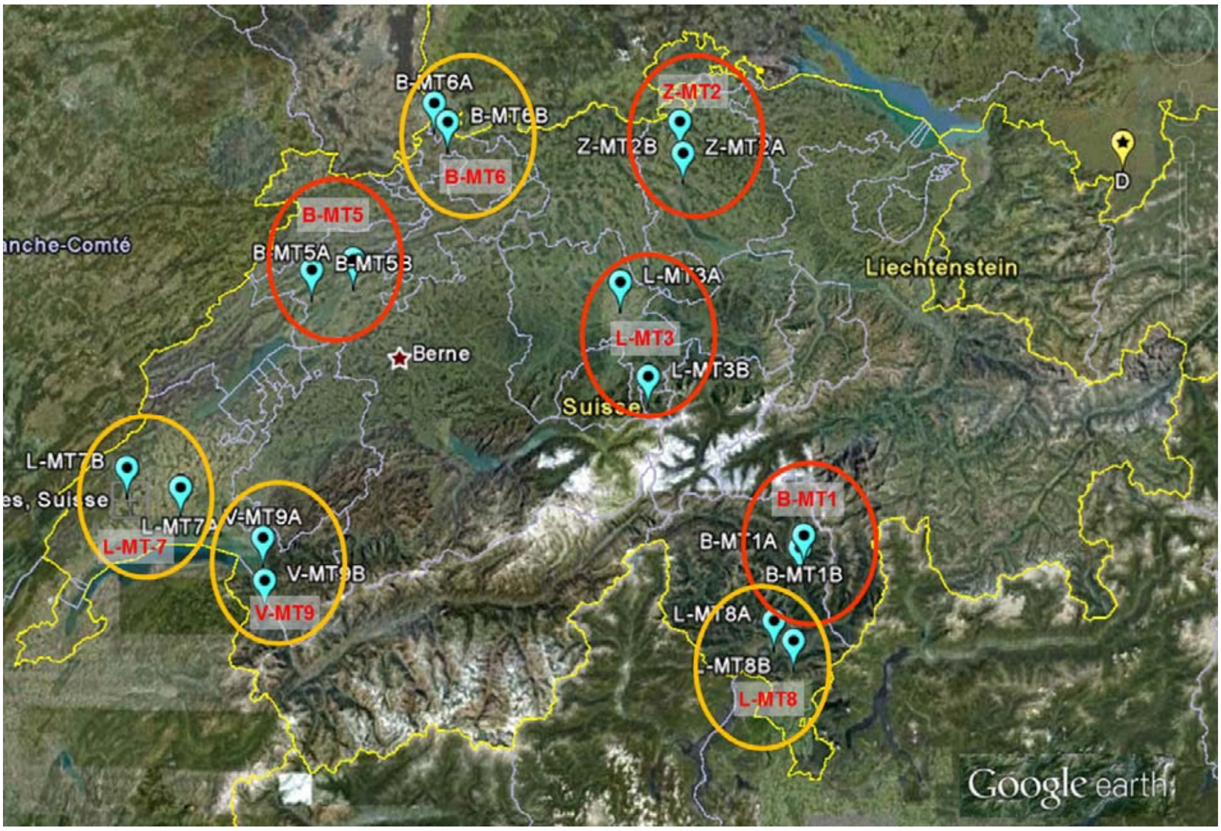
This study was performed in a collaboration with three other Swiss research groups: C. Lengeler/P. Müller and team, Swiss Tropical and Public Health Institute (Swiss TPH), Basel; O. Glaizot and team, Musée Cantonal de Zoologie (MCZ), Lausanne; and O. Petrini/M. Tonolla and team, Istituto Cantonale di Microbiologia (ICM), Bellinzona. IPZ has taken over the project co-ordination, training of investigators and quality control, global data analysis, reporting and result dissemination.

Acquisition of new data based on a specific protocol (2011-2012)

<u>Locations and sites</u>

The spatio-temporal diversity of the mosquito fauna was investigated at eight locations distributed throughout the country and at different altitudes (Fig. 1).

<u>Figure 1.</u> Situation of the selected 8 locations with 2 (nature, suburban) sites each. Red ovals: areas already investigated in 2010 during the pilot study; Orange ovals: new areas.



At each location, two sites were selected, one in suburban/outskirts and one in nature zones, based on land-use GIS maps. These transects were located in: northern Ticino, around Biasca/Malvaglia (MT1); in central Ticino, around Locarno/Taverne (MT8); in the Midplains, around Zürich/Bülach (MT2) and around Lausanne/Saint-George (MT7); in the Inner-Alps, around Luzern/Engelberg (MT3); in Bas-Valais, around Villeneuve/Martigny (MT9): in the Jura, around Bienne/Sonvillier (MT5); and in Basel around Basel/Langenbruck (MT6). Detail on locations and sites with situation maps are given in Annex 1.

Locations/areas are attributed to the 4 teams involved in this study:

- ICM (project leader: *Mauro Tonolla*; in charge of field work: *Evelin Casati*): MT1 (Biasca/Malvaglia), MT8: (Locarno/Taverne)
- IPZ (project leader and global coordinator: *Francis Schaffner*; in charge of field work: *Stefanie Wagner*): MT2 (Zürich/Bülach), MT3 (Luzern/Engelberg)
- MCZ (project leader: *Olivier Glaizot*; in charge of field work: *Elodie Kuhnert*, 2011, and *Sébastien Biollay*, 2012): MT7 (Lausanne/Saint-George), MT9 (Villeneuve/Martigny)
- Swiss TPH (Project leader: *Pie Müller*; in charge of field work: *Tobias Sutter*): MT5 (Bienne/Sonvilier), MT6 (Basel/Langenbruck)

Mosquito collection was performed during 5 periods (July 2011, September 2011, May 2012, July 2012, and September 2012) and over 2 years, to reduce the impact of year-to-year fluctuation due to global weather conditions. For each period, a window of 2 weeks was suggested but most of the time these windows were a bit larger (2.5 to 3 weeks) because of bad weather conditions or practical reasons. At one occasion, two sites were visited only 5 weeks later.

The focus was set on sampling of mosquito immature stages (larvae and pupae), as follows:
- Inspection of potential man-made and natural larval habitats (LH) such as swamps, tree holes, flooded meadows, shallow and standing water bodies, etc. (see Table 3);
- Collection of larvae (also pupae when present) by visual search with a dipper or a net in the water stratum;
- LH units were counted as a “whole LH” (e.g. container) or as “a physical/ecological part of a LH” (e.g. large flooded area) with a maximum size of 20 m²;
- LH units[1] were investigated starting from the central point of the site, spirally outwards, and a minimum of 15 and a maximum of 30 LH units as well as a maximum of 5 LH units per LH category (see Table 3) were sampled;
- Positive, negative (without any mosquitoes) and non-inspected (at the next visit) LH units were reported and described.

In addition, at each visit, adult mosquitoes were trapped overnight at 2 places per site, with a CO_2-baited trap (CDC miniature trap or Biogents Sentinel trap), placed at least one hour before sunset and recovered at least one hour after sunrise.

Geographic coordinates and altitude were collected by using a GPS device (specific or included in a smartphone). Data were collected using the VECMAP system (http://iap.esa.int/projects/health/vecmap) on a smartphone (Android platform, including GPS), or on specific forms, including a field section (sampling) and a laboratory section (identification), and then entered into a data base.

All larval specimens were stored in 70% ethanol and morphologically identified to species level based on state-of-the-art morphological identification tools [8, 9]. Pupae were kept alive in a sample bottle and reared in the laboratory until emergence of adults. These as well as the field collected ones were killed by placing in a deep freezer for at least 15 minutes and morphologically identified based on the previously mentioned tools.

All specimens will be kept at least for 1 year after the end of the study. Some specimens (at least 2 specimens of each species from each location) are kept in the proper way for long-term storage, i.e. larvae mounted on slide or in alcohol (in that case to be mounted later), adults pinned with male genitalia mounted on slide or in alcohol (in that case to be mounted later), and deposited in the reference collection of each institute and/or at a museum.

The study benefits from VECMAP system, mainly for (1) defining the sampling sites (i.e. identifying geo-referenced sampling points/areas in the land-use units), (2) reporting data via a smartphone-to-web system, (3) analysing the distribution data, and (3) modelling the potential distribution of the most common species and mapping species richness in relation to earth observation and climate data. This last task remains to be performed, as the modelling software unit is still in the finalization process.

All data are associated to geo-referenced points and gathered in a central data base, and will be shared with the Centre Suisse de Cartographie de la Faune (CSCF), for edition of distribution maps of bloodsucking insects on the CSCF website.

[1] The minimum number of LH to investigate is defined based on the statement that 70 visits/observations in a defined area may allow to detect more than 90% of the mosquito species [7].

Quality management

Field work was described in a detailed protocol (Annex 2), and a one-day training was organized at the beginning of the project in Ticino for all investigators.

Mosquitoes were identified if possible by each partner and a one-day training was organized for mosquito identification at IPZ, at the end of the first season.

A quality check (for at least 10% of the samples) of the identified samples was performed by an expert at IPZ (F. Schaffner) (see Annex 3 for an example). This included (1) confirmation of the identification of all samples of new species for a region, (2) identification of doubtful diagnostics, and (3) checking of at least 10% of the samples, randomly selected. As not all samples could be identified by the partners themselves, mainly due to the involvement of temporary workers, the percentage of samples checked or identified by the expert varied from 25 to 100%.

2. Results

Potential larval habitats (LH) and the mosquito fauna were investigated at eight locations and at two sites each (Table 2). The same LHs were repeatedly checked; if this was not possible (e.g. container removed or LH no longer accessible), they were replaced by others, if possible from the same category. A total of 1,362 LH observations are reported, ranging from 59 to 109 per site, with an approximately similar number for both nature and suburban zones (48.8 and 51.2%, respectively). The numbers of collected mosquitoes (immature or adult stages) varied widely among the sites, with more adults being collected in the nature zones and more immature in the suburban zones. Mosquito species diversity was higher in the nature zones.

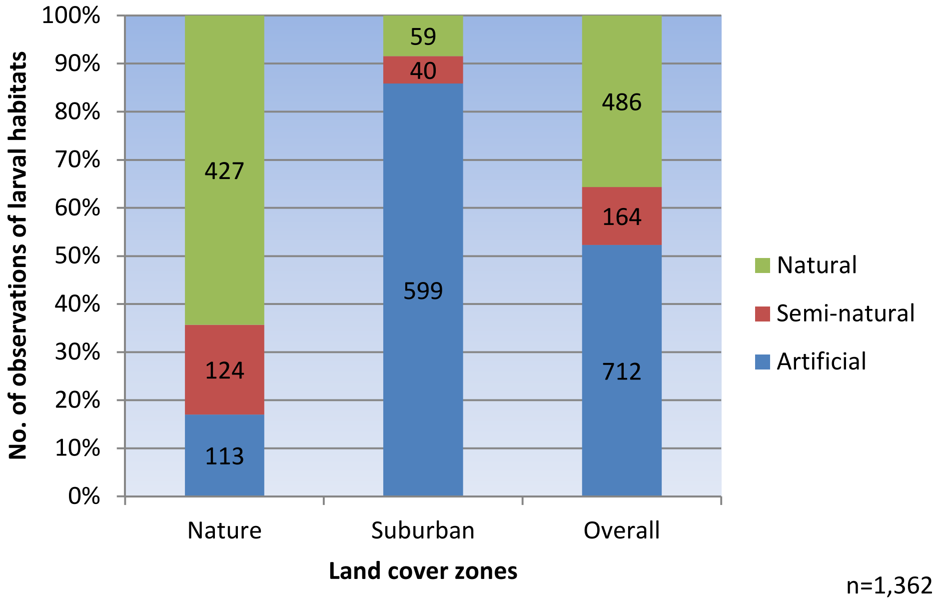
Table 2. Numbers of larval habitats (LH) investigated, mosquitoes collected (larvae and adults) and species identified*, each site (n=16), 2011-12. Adult trapping was performed at 10 occasions at each site. Land cover zones: N, nature; S, suburban.

Locations						No. of observations of			
Canton	**Municipality**	**Place**	**ID**	**Land cover**	**Altitude**	**larval habitats**	**immatures collected**	**adults caught**	**mosquito species**
BE	Biel/Bienne	Im Ried	B-MT5A	S	445	75	928	65	8
BE	Prêles	Châtillon	B-MT5B	N	805	85	528	6	6
BL	Arlesheim	Öli	B-MT6B	N	315	59	217	6	8
BL	Binningen	St Margreten	B-MT6A	S	375	75	925	33	6
LU	Luzern	Friedental	L-MT3A	S	440	106	773	177	7
OW	Engelberg	Gerschnialp	L-MT3B	N	1'255	82	661	0	4
TI	Biasca	Quaresima	B-MT1A	S	295	85	355	30	4
TI	Camignolo	Camignolo	L-MT8B	S	455	75	129	10	5
Ti	Locarno	Bolle di Magadino	L-MT8A	N	198	108	52	546	10
TI	Malvaglia	Lagiüna	B-MT1B	N	360	85	387	483	9
VD	Lausanne	Montoie-Bourdonette	L-MT7A	S	395	109	844	305	5
VD	Mollens	Fermens	L-MT7B	N	675	81	169	80	10
VD	Noville	La Tronchenaz	V-MT9A	N	375	88	257	181	14
VS	Collombey-Muraz	Muraz	V-MT9B	S	415	93	597	21	5
ZH	Oberglatt/Winkel	Hell	Z-MT2B	N	420	76	981	124	11
ZH	Zürich	Irchel	Z-MT2A	S	490	80	1'162	0	3
					Total Nature	***664***	***3'252***	***1'426***	***22***
					Total Suburban	***698***	***5'713***	***641***	***15***
					Grand total	**1'362**	**8'965**	**2'067**	**22**

* Species numbers include 3 pairs of sibling species and 1 species complex (*An. maculipennis*).

Overall, the artificial (man-made) LHs are dominant (50.4%) over natural and semi-natural LHs (37.1 and 12.5%, respectively) (Fig. 2, Table 3). They account for more than 84% of the larval habitats in suburban zones, whereas natural LHs account for more than 65% in nature zones. Within artificial LHs, catch basins, rain water barrels and plastic buckets are dominant (22.3, 19.0 and 12.7%, respectively). Within natural LHs, ponds, large pond borders, swamps and flooded puddles in meadows account together for more than 81% (26.7, 23.9, 15.4, and 15.6%, respectively).

Figure 2. Number of observations of larval habitat types, per land cover zone and overall, all sites, 2011-12. Artificial = both substratum and source man-made; Semi-natural = substratum natural but source man-made (and usually with significant impact of nature, e.g. presence of vegetation); Natural = both substratum and source natural (and reduced direct impact of human activities).

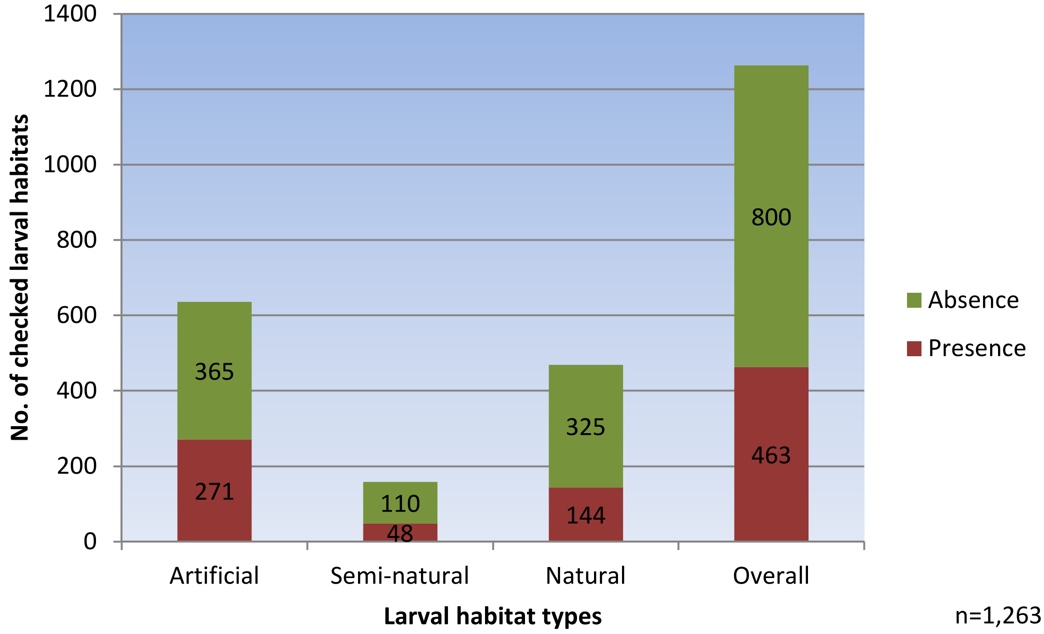


Among the 1,362 LHs inspections, 36.7% revealed the presence of mosquito immature stages (larvae or pupae), with a higher proportion in artificial LHs (42.6%) compared to natural and semi-natural LHs (30.7 and 30.4%, respectively) (Table 3, Fig. 3).

Table 3. Types and numbers of potential larval habitats (LH) and presence/absence of mosquito larvae, per land cover zone, all sites (n=16), 2011-12. P: mosquito present; A: absent.

Larval habitat type and description	Nature zone P	Nature zone A	Nature zone *Total*	Suburban zone P	Suburban zone A	Suburban zone *Total*	Grand Total
Artificial							
Vase				24	26	***50***	50
Flower pot dish				2	13	***15***	15
Bucket, metal		2	***2***	7	10	***17***	19
Bucket, plastic	4	2	***6***	24	51	***75***	81
Pot, concrete				5	3	***8***	8
Container, concrete	2	1	***3***	4	8	***12***	15
Catch basin	4	11	***15***	64	63	***127***	142
Basin				5	8	***13***	13
Fountain	1	4	***5***	34	31	***65***	70
Drinking trough	6	16	***22***	1	6	***7***	29
Rain water barrel	10	5	***15***	55	51	***106***	121
Roof gutter				1	4	***5***	5
Tarpaulin		32	***32***	12	17	***29***	61
Trailer	2		***2***				2
Tyre				4	1	***5***	5
Semi-natural							
Puddle	41	77	***118***	4	6	***10***	128
Rock pool				3	27	***30***	30
Natural							
Ditch	9	16	***25***				25
Pond	22	49	***71***	13	41	***54***	125
Large pond border	40	67	***107***	1	4	***5***	112
Puddle, meadow	13	60	***73***				73
Flooded meadow	1	2	***3***				3
River bed	2	18	***20***				20
Small stream	9	11	***20***				20
Stream puddle		4	***4***				4
Swamp	30	42	***72***				72
Tree hole	4	11	***15***				15
Grand Total	200	430	***630***	263	370	***633***	**1'263**

Figure 3. Number of reported presence/absence of mosquito immatures (larvae or pupae) in potential LHs checked, per LH type, all sites, 2011-12.



Overall, a total of 11,032 specimens (immature and adult stages) belonging to 15 mosquito species, 3 sibling species (of which all 6 species were confirmed) and to the *An. maculipennis* complex were collected (total 22 species; Table 4). The nature zones showed the highest diversity (all species observed) but a lower relative abundance, accounting for 42.4 % of the individuals, whereas the suburban zone revealed only 15 species (including 2 sibling species), but 57.6% of the individuals. Mosquitoes were found at all sites (range 139 - 1,149).

The most abundant species was *Cx. pipiens/torrentium* (not distinguishable at larval stage; 61.2% of all specimens collected) occurring at all except two sites. Six species were frequently observed, i.e. *Cx. hortensis*, *Ae. japonicus*, *An. maculipennis* complex members, *Ae. vexans*, *Ae. cinereus/geminus* (both species, not distinguishable as larva or adult female), and *Ae. sticticus,* accounting each for 8.5 to 3.9% of the individuals. The remaining species were rarely found, accounting each for less than 1%.

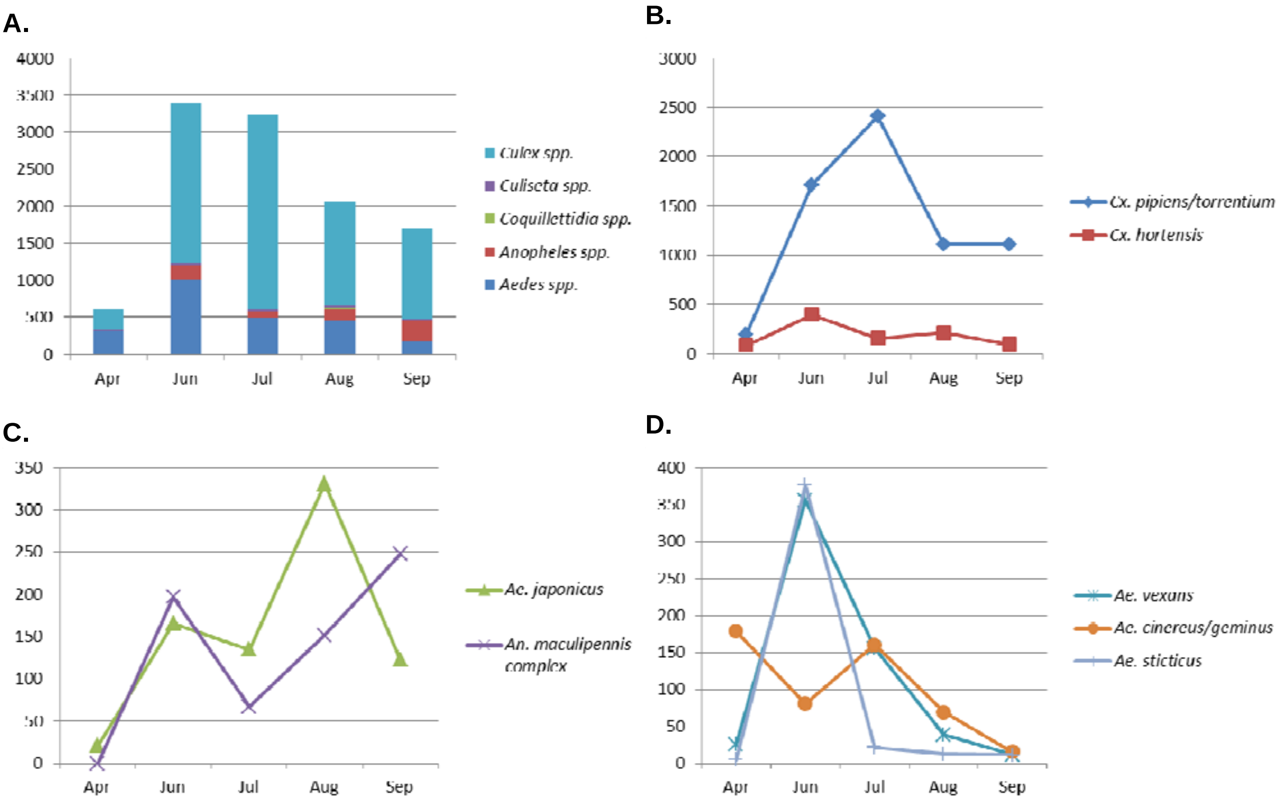
Table 4. Numbers of immature and adult individuals collected, per land cover zone, all sites, 2011-12. (*Sibling species pairs and members of the Maculipennis complex are counted together, as not all specimens have been identified to species level yet).

Mosquito species observed	Nature zone		Suburban zone		Overall		
	Immatures	*Adults*	Immatures	*Adults*	Immatures	*Adults*	Grand Total
*Aedes annulipes/cantans**	55	*5*	0	*1*	55	*6*	**61**
Ae. caspius	0	*1*	0	*0*	0	*1*	**1**
*Ae. cinereus/geminus**	341	*174*	0	*3*	341	*177*	**518**
Ae. geniculatus	1	*1*	1	*9*	2	*10*	**12**
Ae. japonicus	92	*0*	684	*0*	776	*0*	**776**
Ae. punctor	1	*0*	0	*0*	1	*0*	**1**
Ae. rusticus	47	*1*	0	*0*	47	*1*	**48**
Ae. sticticus	62	*366*	0	*1*	62	*367*	**429**
Ae. vexans	126	*455*	0	*8*	126	*463*	**589**
Anopheles claviger	6	*5*	0	*0*	6	*5*	**11**
An. maculipennis complex*	556	*105*	3	*0*	559	*105*	**664**
An. plumbeus	29	*2*	8	*9*	37	*11*	**48**
Coquillettidia richiardii	0	*11*	0	*8*	0	*19*	**19**
Culex hortensis	31	*0*	912	*0*	943	*0*	**943**
*Cx. pipiens/torrentium**	1'780	*297*	4'074	*600*	5'854	*897*	**6'751**
Cx. territans	36	*0*	1	*0*	37	*0*	**37**
Culiseta annulata	77	*1*	5	*2*	82	*3*	**85**
Cs. longiareolata	12	*0*	25	*0*	37	*0*	**37**
Cs. morsitans	0	*2*	0	*0*	0	*2*	**2**
Total	3'252	*1'426*	5'713	*641*	8'965	*2'067*	**11'032**

A total of 160 CO_2-baited trapping nights were implemented, and 70 of them provided a total of 2,067 mosquito specimens (Table 2, Table 4). The nature zone showed the highest relative abundance, with 69.0% of the individuals, whereas only 31.0% were caught in the suburban zone. The highest number of adult mosquitoes was obtained at site MT8A, Locarno (Bolle di Magadino) (Table 2), in nature zone, with more than 60% of the mosquitoes belonging to either *Ae. vexans* or *Ae. sticticus*. The second site in terms of adult relative abundance was MT1A, Malvaglia (Lagiüna), also in nature zone, with the two species mentioned above almost reaching 85% of the collected adults. The third site was MT7A, Lausanne (Montoie-Bourdonette), in suburban zone, with *Cx. pipiens/torrentium* accounting for more than 98% of the caught adults. At two sites, one in nature zone, MT3B, Engelberg (Gershnialp) and on in suburban zone, MT2A, Zurich (Irchel), no adult mosquitoes were caught at all with the CO_2-baited traps.

Highest relative abundances of the mosquito fauna, all species, were observed in June and July, for both nature and suburban zones (Fig. 4). Container-breeding mosquitoes showed either a peek in June (*Cx. hortensis*), July (*Cx. pipiens/torrentium*) or August (*Ae. japonicus*). The floodland/marshland mosquitoes *Ae. vexans* and *Ae. sticticus* peeked in June, but not *Ae. cinereus/geminus*. Among the less abundant species (not shown), *Cq. richiardii* showed a relative short presence period (June-August), with a peek in June, whereas *Ae. annulipes/cantans* and *Ae. rusticus* showed a peek early in the season (April), and *An. plumbeus* late in the season (September).

Figure 4. Seasonal relative abundance of mosquitoes (both immatures and adults) for each sampling period (n=5), all sites, 2011-12. A. All species, grouped by genus. B. The two most abundant species *Cx. pipiens/torrentium* and *Cx. hortensis*. C. Two frequently collected species, *Ae. japonicus* and *An. maculipennis* complex. D. Three other frequently collected species, *Ae. vexans*, *Ae. cinereus/geminus*, and *Ae. sticticus*.



3. Discussion

The consolidated list of Swiss mosquitoes currently comprises 36 species [6], of which eight species belonging to three sibling species pairs and to one species complex were not systematically distinguished in this study (*Ae. annulipes*/*cantans*, *Ae. cinereus*/*geminus*, *An. maculipennis* complex, *Cx. pipiens*/*torrentium*). However, all members of the sibling pairs could be identified in the study by morphology at one stage (male), together with 15 other species, resulting in the observation of a total of 21 species and one species complex (which will be further characterized by molecular analysis).

Compared to the Pilot Study (2010) which relied on single samplings in August/September at five locations, seven additional species were collected in the present study in which eight locations were repeatedly sampled covering the whole season. Of these seven species, three occur strictly (*Ae. rusticus*, *Cs. morsitans*) or mainly (*Ae. punctor*) early in the season (when no investigations were done in the 2010 study) or at sites (single sites only for each species) that were not investigated in 2010. A single adult of one additional species (*Ae. caspius*) was caught at a site that was not investigated in 2010. Another species, *Cq. richiardii*, was collected as adults in both new and former sites (four sites). The species relies on LHs in wetlands and is almost impossible to collect as larvae, and occurs as adult (single generation) only over a few summer weeks. Two species (*Cs. annulata*, *Cs. longiareolata*) were collected at both new and former sites, and the increasing number of sampling could explain the specific finding in this study.

Thirteen species from the Swiss mosquito list were not found during this study: *Ae. albopictus*, *Ae. cataphylla*, *Ae. communis*, *Ae. dorsalis*, *Ae. excrucians*, *Ae. flavescens*, *Ae. pullatus*, *Ae. refiki*; *Cq. buxtoni*, *Cx. modestus*, *Cx. martinii*, *Cs. fumipennis*, *Cs. alaskaensis*. About the reasons why they escaped their detection in this study can be speculated as follow:

- *Ae. albopictus*: this invasive mosquito is spreading into Switzerland from northern Italy; to date, it is only reported from southern Ticino [10], and is not known to be present at the time of the study at the four sites investigated in Ticino.
- *Ae. cataphylla*, *Ae. communis*, *Ae. pullatus*: these three snow-melt mosquitoes occur mainly at high altitudes in the Alps and the Jura [6]. We had expected to find them (in particular *Ae. communis*) in this study at some nature sites, and the reason for their absence remains unclear.
- *Ae. refiki*: this other snow-melt mosquito with strictly only one generation per year is scarce in Europe and is found only at a limited number of places [9, 11]. More sampling in spring has to be performed over the country to detect this species in Switzerland.
- *Ae. dorsalis* and *Ae. flavescens* breed mainly in halophilic LH; both species have been collected only at one occasion in Switzerland [11]. Specific investigations should be performed around salt extraction sites (Aargau, Basel-Landschaft, and Vaud cantons) to determine whether suitable larval habitats are still available in Switzerland.
- *Ae. excrucians*: this species complex is rarely found in central Europe, but known to occur nearby one of our sites (MT2B) [6]. The absence of flooding in 2012 of the known LH and the performance of trapping late in the season in 2011 could explain the non-detection in this study. However, the species cannot be distinguished from *Ae. annulipes/cantans* as adult, and therefore molecular analysis should be performed to confirm its absence.
- *Cq. buxtoni*: As for *Cq. richiardii*, larvae live submerged and are therefore almost impossible to collect. Adults are found together with *Cq. richiardii* but always in very low numbers compared to *Cq. richiardii*. So far, the species has only been detected as adults in southern Ticino [12], nearby one of our sites (MT8-A), but more trappings will probably be needed in July-August to collect the species.
- *Cx. modestus* was reported so far only once in Switzerland, from Ticino [12], but it is not uncommon in the neighbouring French regions Rhône-Alpes [13] and Alsace (F. Schaffner, personal observations). Specific searches in favourable environments (e.g. reed beds) might lead to new findings of this species which is regarded as the main vector of West Nile virus in some European wetlands such as the Camargue [14].
- *Cx. martini*, *Cs. fumipennis*, *Cs. alaskaensis.* These rare species in central Europe have been collected so far at few places only in Switzerland [6, 11, 15], but more samplings would probably allow to identify new places.

In this study, some differences in immature sampling and adult trapping can be pinpointed:

- Three species caught as adults were not collected as immatures: *Ae. caspius*, caught as a single specimen, probably flying from a distant LH (not sampled in the study); *Cq. richiardii* which breeds in permanent water with immature stages submerged and fixed on plant stems (and not surfacing for breathing) and therefore almost impossible to collect as larvae except by using specific techniques; and *Cs. morsitans*, of which two females were caught in Mollens, August 2012. This species is abundant in early spring only [8] but a second generation, reduced in number, can be develop at the end of the summer (F. Schaffner, personal observation).
- Five species collected as immatures were not caught as adults: *Cx. hortensis*, *Cx. territans*, *Cs. longiareolata*, which are known to be not or only very weakly attracted to CO_2-baited traps used in the study. Similarly, *Ae. japonicus* is only well attracted to CO_2-baited traps when using an additional chemical lure [16]. *Aedes punctor* is known to be well attracted but most probably is very scarce in the surveyed sites (and a single immature specimens was collected).

The experimental approach (larval sampling and CO_2-baited trapping) looks reliable for collecting most of the mosquito species, and in particular the vector species. Association of larval sampling and adult trapping has allowed to enlarge the collected species spectrum, as eight species among 22 were collected by only one of the two methods. Only rare species, which because of their scarcity might have no or little impact on mosquito-borne disease transmissions, were not collected in our study. As for invasive mosquitoes (*Ae. albopictus*, *Ae. japonicus*), additional trapping techniques increase the chances to detect their presence and estimate their abundances, by using ovitraps, infusion-baited gravid traps, or BG-Sentinel™ traps with Traptech™ lure [16, 17]. However, modelling the distribution of the mosquito species remains to be performed in order to validate the experimental design for risk mapping.

In this study, we performed, per location, five investigations at two sites instead of only one at five sites as in the Pilot Study, 2010. When comparing the results in terms of species present at sites that were investigated during both studies (Table 5), congruent results were obtained in 26 instances (XX in Table 5). The present study identified the presence of mosquito species in 25 instances more than the Pilot Study, whereas the inverse occurred in 3 cases only. When comparing the results at locations (all 4 sites in the Pilot Study, 2 in this study, per location), mosquito species were detected in both studies in 22 cases, and a species was found only in this study in 18 cases, whereas the inverse happened in 5 cases only (data not shown). This confirms the higher sensitivity of the field experimental design of this study, as compared to the Pilot Study.

<u>Table 5.</u> Mosquito species detected at sites investigated in both the Pilot Study (2010) and this study (2011-12). Species detection: X, in Pilot Study only; XX, in both studies; XXX, in this study only.

Mosquito species observed	**Nature zone**								**Suburban zone**							
	B-MT1B		**B-MT5B**		**L-MT3B**		**Z-MT2B**		**B-MT1A**		**B-MT5A**		**L-MT3A**		**Z-MT2A**	
	2010	**2011-12**	**2010**	**2011-12**	**2010**	**2011-12**	**2010**	**2011-12**	**2010**	**2011-12**	**2010**	**2011-12**	**2010**	**2011-12**	**2010**	**2011-12**
Aedes annulipes/cantans	X							XXX				XXX	X			
Ae. caspius																
Ae. cinereus/geminus		XXX						XXX								
Ae. geniculatus											XX	XX				
Ae. japonicus						XXX		XXX			XX	XX	XX	XX	XX	XX
Ae. punctor		XXX														
Ae. rusticus																
Ae. sticticus		XXX					XX	XX						XXX		
Ae. vexans	XX	XX					XX	XX		XXX		XXX	XX	XX		
Anopheles claviger	X		XX	XX			XX	XX								
An. maculipennis complex		XXX	XX	XX			XX	XX								XXX
An. plumbeus												XXX	XX	XX		
Coquillettidia richiardii								XXX						XXX		
Culex hortensis	XX	XX		XXX				XXX		XXX		XXX	XX	XX		
Cx. pipiens/torrentium	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
Cx. territans	XX	XX	XX	XX			XX	XX		XXX						
Culiseta annulata						XXX						XXX				
Cs. longiareolata				XXX												
Cs. morsitans																XXX
Total no. of species	6	8	4	6	1	3	6	11	1	4	3	8	6	7	2	4

Considering the geographical distribution of the collected species, solid conclusions can only be stated for *Ae. japonicus* which was collected in high numbers at all places in northeaster Switzerland, but not in western and southern Switzerland. This confirms that this species had been introduced in northern Switzerland and was spreading from there [18]. The most westerly confirmed occurrence in Switzerland is Biel, and is not clear why the species, which rapidly spread after its introduction [18] is not invading western Switzerland. The recently published presence of this mosquito in Germany at two places (around Stuttgart and Bonn [19]) therefore might originate from separate introductions, not from unnoticed continuous expansion of the Swiss population northwards.

Other data suggesting that some species might have limited geographical distributions are too weak for any conclusions to be drawn (except for *Ae. albopictus* which is restricted to southern Ticino which was outside our study area). Indeed, *Ae. caspius* and *Ae. punctor* were found only southern to the Alps, and *Cs. morsitans* only in Western Switzerland, but the two last species are known to also occur in other regions of the country. All other species do not show any particular distribution.

All 22 mosquito species were found in nature zone, and five of them (*Ae. caspius*, *Ae. punctor*, *Ae. rusticus*, *An. claviger*, and *Cs. morsitans*) where not found in the suburban zone. However, four additional species (*Ae. cantans/annulipes*, *Ae. cinereus/geminus*, *Ae. vexans*, and *An. claviger*) were found in the suburban zone only as adults, and therefore could have flown into these sites from distant LHs. These results confirm the higher diversity in nature zone as compared to suburban zone, already suggested in our Pilot Study.

From collected data, seasonal dynamic tendencies can be suggested a follows:

- *Ae. vexans* and *Ae. sticticus* breed in wetlands and are usually abundant after flooding (from spring to fall); in our study, they were highly abundant in June (Fig. 4D). *Aedes cinereus* and *Ae. geminus* have similar LHs and therefore could be expected to show a similar dynamic, but this was not the case in this study. Rather, their populations were more abundant in April and July, with less marked peaks of abundance. All four species can fly over some distances to disperse and seek hosts.
- *Ae. japonicus*, which was shown to be a vector under laboratory conditions for a number of pathogens, including West Nile and Dengue virus [20-24], was more abundant in summer (August; Fig. 4C), when high temperatures would favour virus amplification, thus rendering this species a potential vector. When established for several years, the species seems to become the most abundant one in the suburban zone, as shown at Zürich, Irchel, where it was more abundant than *Cx. pipiens/torrentium* in three of the five investigated periods (but the Culex species prevailed overall).
- Among malaria vectors, *An. plumbeus* whose populations have increased over the last years and which was shown to be an efficient vector [25], becomes abundant late in the season, in August-September, whereas species form the Maculipennis complex were abundant over the whole summer, from June to September (Fig. 4C).
- *Cq. richiardii* shows one peak in June which is in agreement with its univoltine characteristic.
- *Ae. rusticus*, *Ae. cantans*, and *Ae. annulipes* were mostly found in April, confirming their classification as spring mosquitoes with only one generation (*Ae. rusticus*) or showing occasionally a weak second generation or staggered secondary cohorts.
- *Cx. pipiens* and *Cx. torrentium* (Fig. 4B) show a weak population in spring, which corresponds to the first generation produced by the overwintering females; later, populations are abundant all over the summer and fall, in particular in June and July; they are the most abundant mosquitoes at almost all places, except two nature locations (Locarno, Bolle di Magadino and Noville, La Tronchenaz).

Overall (Fig. 4A), the highest abundance of mosquitoes occur in a period less favourable for pathogen transmission (June, lower temperature), but high abundance were observed in July, which looks to be the highest risk period with regard to vector abundance and suitability of pathogen replication.

Some in-depth investigations remain to be performed: molecular identification within complexes and sibling species (larvae and females of *Ae. cinereus/geminus*, *Ae. annulipes/cantans*, *Cx. pipiens/torrentium;* larvae and adults of the Maculipennis complex) by PCR or MALDI-TOF MS technique (recently established by IPZ and Mabritec, Riehen [26, 27]), together with samples of the Pilot Study 2010); statistical analysis; and finally extrapolation and modelling for risk mapping using the VECMAP modelling component which currently is in the finalization process.

4. Conclusions

The chosen approach to characterize the spatio-temporal diversity of the mosquito fauna in Switzerland, repeated larval sampling and CO_2-baited trapping at eight nature and suburban locations, was successful for collecting most of the Swiss mosquito species, including the vector species. From the known 36 species from Switzerland, only 13 were not identified in the collected mosquitoes, mainly species known to be rare or having halophilic or thermophilic requirements. The seven most common mosquitoes (*Ae. cinereus/geminus*; *Ae. japonicus*, *Ae. sticticus*, *Ae. vexans*, *An. maculipennis* complex, *Cx. hortensis*, *Cx. pipiens/torrentium*) accounted for around 97% of the collected specimens, all but *Cx. hortensis* (second most common) being described as having a potential to act as vector (Table 1). As virtually no adults of these vector species were collected in suburban areas (with the exception of *Cx. pipiens/torrentium*), specific vector surveillance could abstain from using adult collection in these areas. Laboratory vector competence studies under realistic Swiss climate conditions are required to assess the vector competence of the Swiss mosquito populations, and such studies are currently being performed at IPZ (Zürich) for West Nile virus.

Morphological identification of mosquitoes is in many instances a time-consuming and sometimes difficult task, and the capacity for large scale surveillances is not available in Switzerland (identification was often done by temporary, semi-skilled collaborators, requiring extensive quality control). The recently developed MALDI-TOF MS database, currently containing spectra of immature and adult stages of 35 European species [27], can be used as a high-throughput, cost-efficient and highly reliable identification tool. The technique also is suitable to identify several species in pools of eggs, and thus is particularly useful for the surveillance of invasive container breeding *Aedes* species. *Aedes albopictus* was not detected in the study (the known distribution area in southern Ticino was not included) but is expected to spread further. Indeed, very few specimens (adults or larvae) of *Ae. albopictus* have repeatedly been detected in summer in Germany and Austria north of the Alps [28, 29].

The invasive species *Ae. japonicus* was overall the third most common species, being the prevailing species in late summer. To answer the question whether this species is a threat to biodiversity by reducing resident container breeding species requires further investigations. In North America, where the species has also been introduced, this issue is controversially discussed [30-32].

Finally, as already stated in the Pilot Study, a nation-wide surveillance could be extended to include also other arthropod vectors (sand flies, biting and sucking flies, ticks). More knowledge on vector distribution and capacity in Switzerland will allow to develop a risk assessment and management for vector-borne diseases that might emerge under environmental and climate changes.

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