Scuola universitaria professionale della Svizzera italiana
Dipartimento ambiente costruzioni e design
Laboratorio microbiologia applicata

SUPSI

Final Report

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Project title: **Inventory of *Culicidae* in and around the nature reserve "Grande Cariçaie" 2019**

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1 Introduction

The family of Culicidae (Diptera, Nematocera) comprises more than 3'600 known mosquito species, divided into the subfamilies Anophelinae and Culicinae (Becker et al., 2010; Roskov et al. 2019). Mosquitoes can adapt to a wide range of habitats and therefore occur throughout the world, although approximately three quarter of them live in humid tropic and subtropic areas (Becker et al., 2010). They lay their eggs in water or humid soil and are able to exploit a multitude of different water bodies ranging from permanent and clean water bodies to polluted or tiny water accumulations. Eggs hatch to become larvae, which develop into several larval stages and then change into pupae. Finally, the adult mosquito emerges from the pupa. The life history strategies vary between species. Females of most species require a blood meal for the development of eggs, but host preferences differ. During the blood feeding pathogens can be transmitted from the mosquito to the host and vice versa (Becker et al. 2010).

Still today, mosquitoes are a major global public health concern, as they can transmit dangerous pathogens. In Switzerland, after the eradication of endemic Malaria in Europe in the first half of the 20th century (Becker et al. 2010), the significance of mosquitoes has been restricted to their role as nuisance and as vector of a few pathogens of veterinary importance (Schaffner & Mathis, 2013). Only recently, with (re-)emergence of pathogens of potential human importance in neighboring countries (ECDC, 2020; Semenza & Zeller, 2014) as well as the spread of invasive mosquito species in Switzerland (Swiss Mosquito Network, 2019), the focus is shifting again.

Nonetheless, studies giving an overview of the Swiss mosquito fauna are rare (Flacio et al. 2014; Flacio et al. 2015). A consolidated list of 36 different species of Culicidae in Switzerland was published in 2013 (Schaffner & Mathis, 2013). The databank of the Swiss Centre for Fauna Cartography (CSCF) lists 23 species and is currently being revised (info fauna, 2017). Also, in the nature reserves of the Grande Cariçaie little is known about the faunistic state of Culicidae. Single entries from 1988 in the official database of the "Association de la Grande Cariçaie", giving records of *Aedes communis* and *Culex pipiens* in the reserve "Grèves de la Motte" are the only documented detections.

Therefore, for the first time an extensive study on Culicidae was carried out in this area. The goals of the study were the following: first, to monitor diversity of Culicidae in the nature reserve "Grande Cariçaie", secondly to investigate if the mosquito fauna from the nature reserve can induce nuisance on surrounding settlements and thirdly, to generally enlarge data on wetland mosquito species in Switzerland. Similar studies have been carried out in nature reserves in the Canton of Ticino (Flacio et al., 2014; SUPSI, 2019a; SUPSI, 2020 (in progress)).

2 Material and Methods

2.1 Sampling site

The nature reserve “Grande Cariçaie” is an area of almost 3’000 ha along the south and east shore of the lake Neuchâtel. It consists of eight different nature reserves which belong to the cantons of Vaud, Fribourg and Neuchâtel. All together it is the biggest wetland area in Switzerland. It provides a sanctuary for approximately 800 plant and 10’000 animal species and is protected by the Ramsar convention (Association de la Grande Cariçaie, 2019; The Ramsar Convention Secretariat, 2014). The city of Yverdon-les-Bains with 30’211 inhabitants (Canton de Vaud, 2018) is located at the southern border of the study area. The remaining area is characterized by small settlements, agricultural land and the different parts of the nature reserve. Lake tourism is an important sector in the region.

2.2 Sampling locations

In total 15 sampling sites were investigated. Of those 13 sampling sites were located in the different nature reserves and two were situated outside of the nature reserves (Yverdon 1, Yvonand 4). Eight sites were in a completely natural environment, six sites were in the immediate vicinity of peri-urban zones and one site was in an urban area. Site 5 had to be moved 200 m for reasons of inaccessibility for sampling in July, August and September (see Figure 1, also see Annex A1 for coordinates of sites).

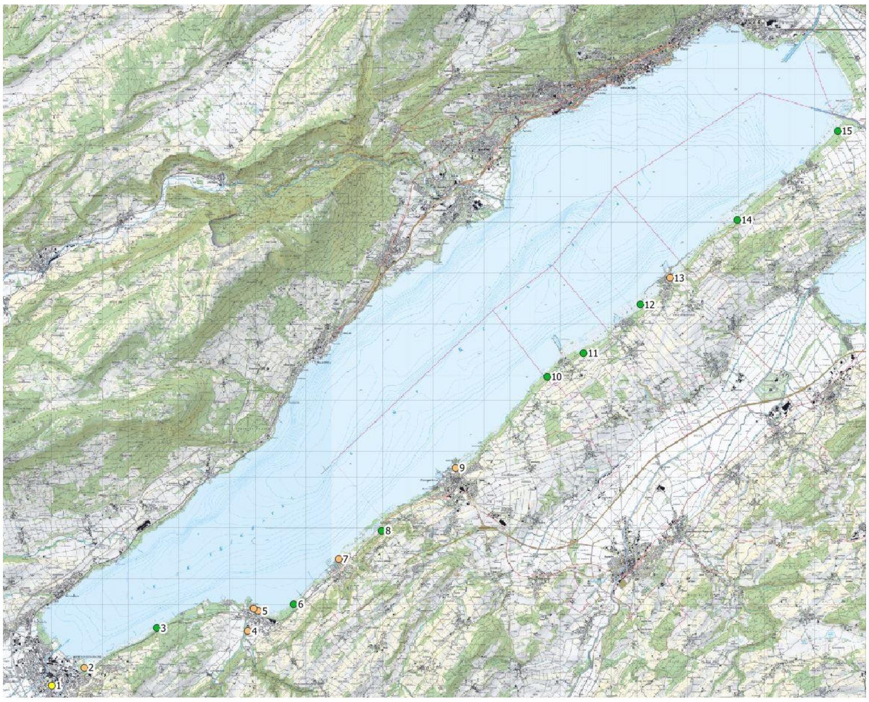

Figure 1: Sampling sites 1 - 15. The urban site is yellow, the peri-urban sites are orange and the sites in the nature reserves are green.

2.3 Sampling methods

To collect adult mosquitoes, Centers for Disease Control (CDC) miniature light traps (model 512, John W. Hock Company, Florida, USA) (Figure 2) were set once a month for 24 h. They were hung up at approximately 1.5 m above ground and were equipped with dry ice in a container to attract female adult mosquitoes. A battery provided energy for the fan that sucks the mosquitoes into the catching bag. Caught mosquitoes were killed by exposure to dry ice and stored at -20°C.

Figure 2: CDC trap with blue dry ice container (Photo: A. Gander).

Larvae were also collected monthly using a standard pint dipper (model 1132H, BioQuip Products, USA) (Figure 3) in surrounding suitable waterbodies. For every sampling site, water collection was repeated at least 3 times. Suitable standing water bodies surrounding the adult trapping sites were checked. Usually dipping was done in the same water bodies. If temporary water bodies fell dry, other surrounding water bodies, if available, were checked as well. In July and August in (peri-) urban sites also artificial water bodies and containers were checked for presence of larvae (examples in Figure 4) and sampled using a plastic pipette.

Figure 3: Larval sampling with dipper (Photos: S. Flämig).

Figure 4: Examples for artificial mosquito breeding sites: rainwater collected in used tyres,and tarps in a marina, garden pots, bird baths or former ponds (ponds with fish or amphibians are not a breeding site!) (Photos: A. Gander).

2.4 Sampling period

The study was carried out monthly between April and September 2019, resulting in six monitoring rounds. Optimal weather conditions consisted of a dry evening without wind. The last round which was planned for mid-September had to be postponed due to strong North east wind. This would have impacted the catching results, as mosquitoes fly less in windy conditions. The last round was finally carried out in the beginning of October (2-3 Oct. 2019) For the purpose of consistency the round was still counted as the “September round”.

The year 2019 was characterized by a spring season which was in the standard of the years 1981-2018, except for the month of May, which was the coldest since the beginning of measurements. The summer was warmer than normal with precipitations close to the standard and early fall was warmer and drier than the standard (MeteoSchweiz, 2019).

2.5 Identification

Mosquitoes were identified to the species level using morphological keys (Becker et al., 2010, Vignes & Lebbe, 2013, Schaffner et al., 2001; Romi et al., 1997). For adult mosquitoes, only females were considered. For larvae, whenever possible third or fourth-instar individuals were sampled. For uncertain cases and as an overall quality check, some individuals were double-checked by further experts (E. Flacio, F. Schaffner).

In several cases, species were grouped as differentiation by morphological characters is very difficult or even impossible and molecular methods were not applied in this study:

- *Anopheles claviger* and *Anopheles petragnani* were grouped as *Anopheles claviger-petragnani.*
- *Anopheles maculipennis* was named *An. maculipennis sensu latu* (s.l.).
- The two biotypes *Culex pipiens pipiens* and *Culex p. molestus* as well as *Culex torrentium* were named *Cx. pipiens-torrentium*.
- *Aedes cinereus* and *Aedes geminus* were grouped as *Ae. cinereus-geminus.*
- *Aedes cantans and Ae. annulipes* were grouped as *Aedes cantans-annulipes* (reliable differentiation only possible with males).

3 Results

3.1 Diversity of mosquito species collected

A list of all species found as adult or larvae is given in Annex A 2 Full list of species. A total of 3'422 adult female Culicidae were collected in six sampling rounds. Of these 3'313 could be identified to the species level. 13 different species were identified (Figure 5). The most abundant were *Ae. cantans-annulipes, Ae. vexans vexans, Ae. sticticus* and *Ae. cinereus-geminus*.

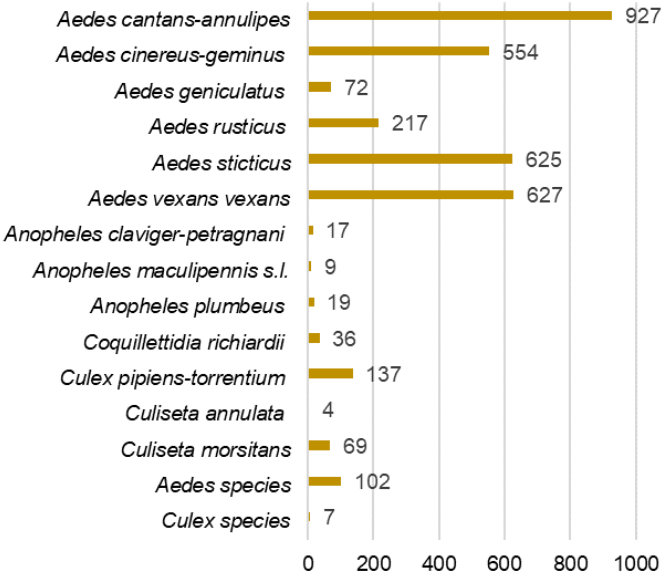

Figure 5: Adult female mosquitoes identified in the study.

During the whole monitoring 252 larvae were sampled. Of the 15 different species identified the most abundant were *Ae. cantans-annulipes*, *Ae. rusticus*, *Cs. morsitans,* and *Ae. cinereus-geminus* (Figure 6).

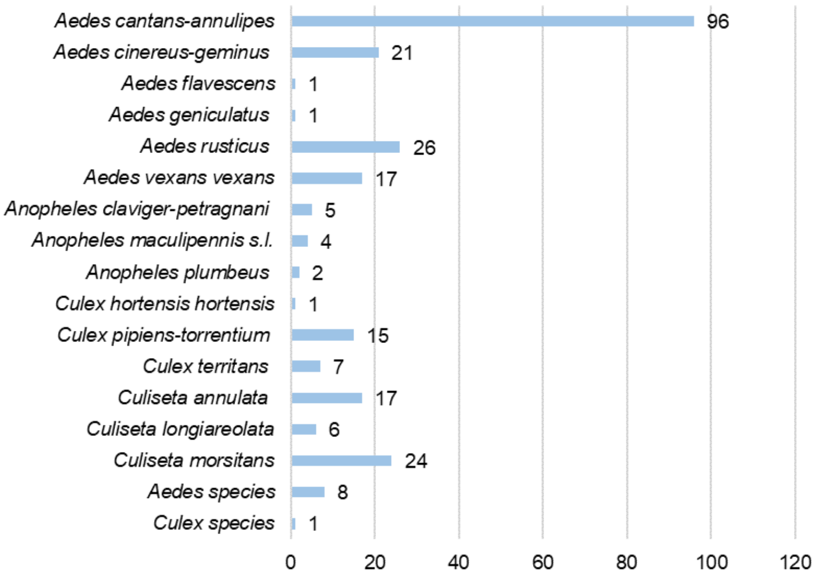

Figure 6: Larvae identified in the study.

3.2 Mosquito species collected in nature and peri-urban areas

3.2.1 Adults

Table 1 gives the numbers for adult females caught per species and site. Catch numbers per site ranged from 11 to 1’306 individuals. Average adult catches per site were higher in nature sites than in peri-urban sites (327.25 ± 431.3 vs. 114.86 ± 92.5). Site 15 (Cudrefin) was the most productive (1’306 individuals), followed by sites 14 (Champmartin) (539) and 11 (Ostende) (296). The most productive peri-urban site was site 7 (Cheyres) (239 individuals) followed by site 1 (Yverdon) (236).

Table 1: Numbers of adult female mosquitoes caught according to species and site.

No. female adults/species/site																
	URB.	PERI-URBAN						NATURE								ALL SITES
	1 site	6 sites						8 sites								15 sites
Species	**1**	**2**	**4**	**5**	**7**	**9**	**13**	**3**	**6**	**8**	**10**	**11**	**12**	**14**	**15**	**1-15**
Aedes cantans-annulipes	109	13	-	20	63	16	43	1	23	113	34	199	4	105	184	927
Aedes cinereus-geminus	5	2	-	7	62	52	14	3	27	5	16	33	4	276	48	554
Aedes geniculatus	-	1	-	2	5	-	10	1	-	2	26	1	-	7	17	72
Aedes rusticus	32	-	1	4	32	13	15	3	4	4	73	8	-	11	17	217
Aedes sticticus	3	2	-	1	22	2	3	-	4	5	12	8	-	36	527	625
Aedes vexans vexans	1	1	-	5	38	19	10	-	5	5	18	16	-	64	445	627
Anopheles claviger-petragnani	-	-	-	-	1	-	-	-	1	11	1	-	1	2	-	17
Anopheles maculipennis s.l.	-	-	1	-	-	6	-	-	1	-	-	-	-	-	1	9
Anopheles plumbeus	-	5	-	6	1	-	1	-	-	-	1	4	-	-	1	19
Coquillettidia richiardii	-	1	1	2	2	8	1	1	2	17	1	-	-	-	-	36
Culex pipiens-torrentium	85	7	8	1	4	-	2	-	-	-	5	9	7	6	3	137
Culiseta annulata	1	-	-	1	1	1	-	-	-	-	-	-	-	-	-	4
Culiseta morsitans	-	-	-	-	1	2	5	5	5	3	12	9	3	23	1	69
Aedes species	-	1	-	2	7	6	1	-	1	4	1	8	1	8	62	102
Culex species	-	2	-	2	-	-	-	-	-	-	1	1	-	1	-	7
Total no. adults/site	236	35	11	53	239	125	105	14	73	169	201	296	20	539	1306	3422

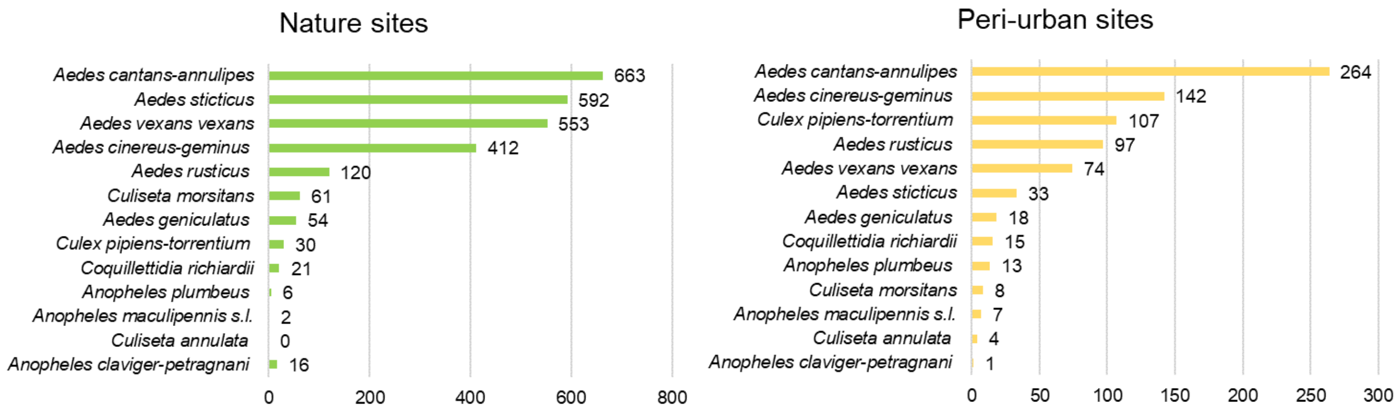

Figure 7: Ranking of adult species according to quantities caught for urban and peri-urban sites. Note that number of sites was 8 in nature, 6 in peri-urban, 1 in urban area. For better visualization, the scales on the x-axis is different for the two graphs.

Figure 7 shows the number of adult female mosquitoes ranked according to quantities caught in nature sites versus urban and peri-urban sites. In all zones *Ae. cantans-annulipes* was the most abundant species.

3.2.2 Larvae

Larval catches per species and site are compared in Table 2. Larval catches per site varied between three and 50 specimens. Average larval catches per site were higher in peri-urban sites than in nature sites (18 ± 17.8 vs. 15.75 ± 5.6). They were highest in peri-urban site 5 (Yvonand) (50 individuals). The highest catch in a nature site was at site 14 (Champartin) with 27 specimens.

Table 2: Numbers of larvae caught according to species and site.

No of larvae/species/site																
	URB.	PERI-URBAN						NATURE								ALL SITES
	1 site	6 sites						8 sites								15 sites
Species	**1**	**2**	**4**	**5**	**7**	**9**	**13**	**3**	**6**	**8**	**10**	**11**	**12**	**14**	**15**	**1-15**
Ae. cantans-annulipes	6	-	-	26	17	1	5	5	8	6	3	2	4	7	6	96
Ae. cinereus-geminus	-	-	-	3	2	2	-	-	9	-	-	1	-	2	2	21
Ae. flavescens	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Ae. geniculatus	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Ae. rusticus	1	-	1	-	4	-	-	1	-	-	6	1	1	7	4	26
Ae. vexans vexans	-	-	-	-	3	-	-	1	-	-	-	-	7	6	-	17
An. claviger-petragnani	-	-	2	3	-	-	-	-	-	-	-	-	-	-	-	5
An. maculipennis s.l.	-	-	-	-	-	-	-	2	-	-	-	-	-	2	-	4
An. plumbeus	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	2
Cx. hortensis hortensis	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Cx. pipiens-torrentium	4	4	-	-	1	1	-	-	-	-	-	5	-	-	-	15
Cx. territans	-	-	-	-	-	3	-	2	-	-	-	-	2	-	-	7
Cs. annulata	-	-	-	14	-	-	2	-	-	1	-	-	-	-	-	17
Cs. longiareolata	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
Cs. morsitans	1	-	-	-	4	1	-	-	-	7	-	2	4	3	2	24
Aedes species	-	-	-	4	2	-	-	-	1	1	-	-	-	-	-	8
Culex species	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Total no. of larvae/species	**19**	**5**	**3**	**50**	**34**	**8**	**7**	**11**	**18**	**15**	**9**	**13**	**19**	**27**	**14**	**252**

Figure 8 shows the numbers larval species ranked according to quantities caught in nature sites versus urban and peri-urban sites. In all zones *Ae. cantans-annulipes* was the most abundant species.

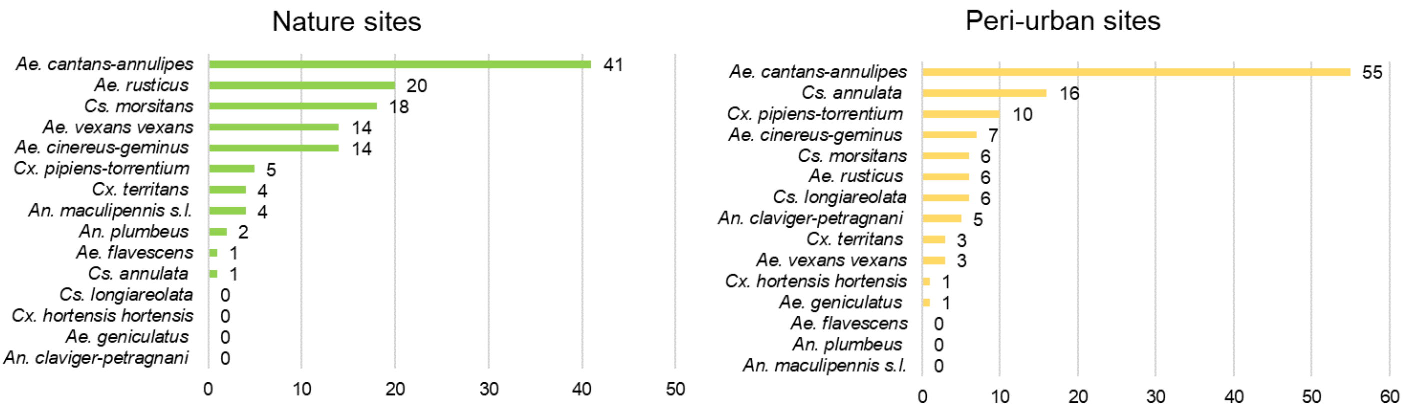

Figure 8: Ranking of larval species according to quantities caught for urban and peri-urban sites. Note that number of sites was 8 in nature, 6 in peri-urban, 1 in urban area. For better visualization, the scales on the x-axis is different for the two graphs.

3.3 Seasonal dynamics

3.3.1 Adults

Figure 9 shows the seasonal dynamics of adult female catches by giving catches per monthly catching round for the five most abundant species and for all other species grouped together. For the full table giving adult catches per individual site see Annex A 2 Full seasonal tables for adults, larvae.

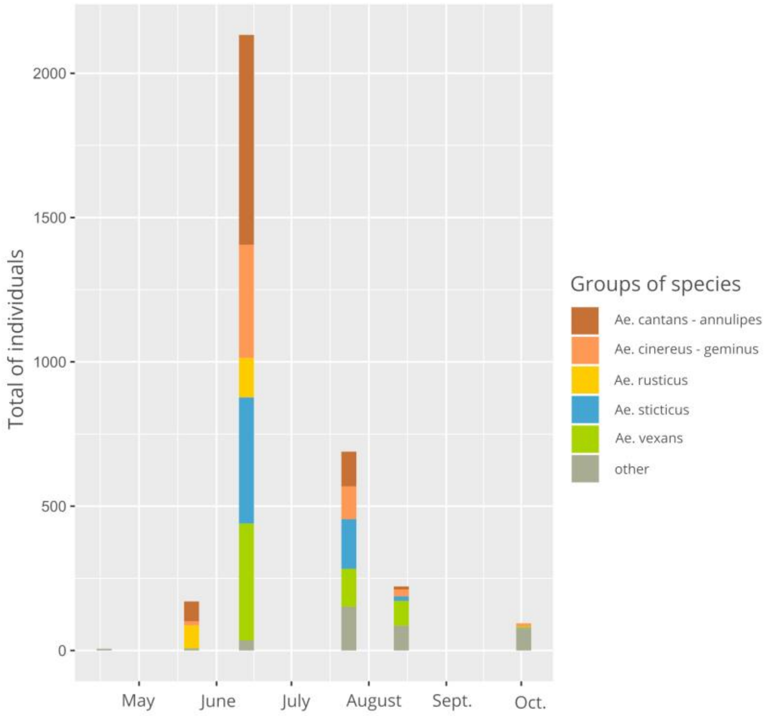

Figure 9: Adult catches per catching round (April - September). The five most abundant species are shown separately, the remaining species are grouped into "other".

3.3.2 Larvae

Fehler! Verweisquelle konnte nicht gefunden werden. shows the seasonal larval dynamics by giving larval catches per monthly catching round for the four most abundant species and for all other species grouped together. For the full table including also larval catches per individual site see Annex A 2 Full seasonal tables for adults, larvae.

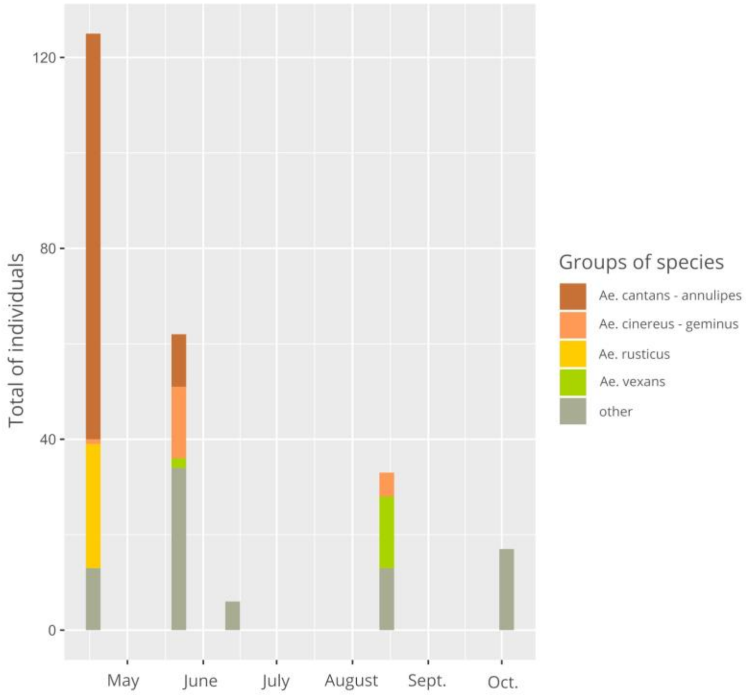

Figure 10: Larval catches per catching round (April - September). The four most abundant species are shown separately, the remaining species are grouped into "other".

4 Discussion

4.1 Diversity and abundance of species

Seventeen different species were detected (caught either as adult or larva or both). For all of Switzerland a total of 36 species has been published in a consolidated list (not including doubtful records of single observations) (Schaffner & Mathis, 2013). With almost half of those species detected in the “Grande Cariçaie”, the nature reserve hosts a rich diversity of mosquito species. Of the two species mentioned in the official database of the “Association de la Grande Cariçaie” from the year 1988, one species - *Cx. pipiens* - was confirmed in our study. It is one of the most common species in Switzerland. The second species *Ae. communis* was not confirmed in our study.

No invasive mosquito species were detected during the whole study. In different parts of Switzerland, the invasive ‘tiger mosquito’ *Ae. albopictus,* the ‘bush mosquito’ *Ae. japonicus* and a third Asian species, *Ae. koreicus* are becoming or are already established (ECDC, 2019a; ECDC 2019b; Flacio et al., 2015; Swiss Mosquito Network, 2019).

The total number of 3’313 adult females and 252 larvae of Culicidae caught in this study is rather low compared to other studied wetland areas (Flacio et al., 2014; SUPSI, 2020 (in progress)). These other areas are dominated by *Ae. vexans* and *Ae. sticticus*. These species are able to find suitable floodplain areas to deposit their eggs in humid soil, where they can survive for at least 5 years (Becker et al., 2010). If the area is flooded and the water temperature exceeds 9°C, the eggs hatch in masses (Becker et al., 2010; Schaffner et al., 2001). In those areas, for example the delta region “Bolle di Magadino” in Canton Ticino or at the “Lac de La Gruyère” in Canton Fribourg the two species have been responsible for great mosquito nuisance in the past, even necessitating treatments against larvae (Flacio et al., 2014; GLZ, 2018; Lüthy, 2014). In our study, *Ae. vexans* and *Ae. sticticus* were the second and third most commonly caught adult species. But their proportion is rather low in contrast to the above-mentioned wetlands. This might be due to the fact that the "Grande Cariçaie" nature reserves do not have extensive floodplains, where large surfaces are flooded at the same time and where unhatched eggs accumulate in the soil over several years. Also, the detailed flooding regime at the Grande Cariçaie might not be suitable for the mass development of the two mentioned species, e.g. concerning length and areas of flooding or fluctuations.

Catching methods differ in their efficiency for certain species and comparisons with other studies should hence be made with caution. As most species in this study were found both as larva and adults, it shows that the methods used for adult and larval sampling worked well in this case. CDC traps and dipping are the most common methods used to catch the majority of species. Nevertheless, certain species could be more common than the results suggest, as they do not respond well to these methods. This could be the case for those *Culex* species which are not mainly attracted to mammals. These are: *Cx. pipiens* form *pipiens* and its sibling species *Cx. torrentium*, which are ornithophilic (Becker et al., 2010; Flacio et al., 2014) as well as *Cx. hortensis* and *Cx. territans* which feed mainly on reptiles, amphibians or also birds (Schaffner et al. 2001). The two latter ones were only detected as larvae in this study. To achieve better catching results for *Cx. pipiens-torrentium*, traps would have to be positioned higher, almost in the trees (pers. communication E. Flacio) This would however impact catching results for other species that do not fly so high. The other biotype *Culex p. molestus* readily bites humans and is therefore also attracted by the CDC traps used.

Another species that could be more common that our larval results suggest is *Cq. richiardii*. Larvae of *Coquillettidia* species are impossible to catch by dipping, as they live in dense vegetation in the water and are attached to water plants with their siphon. Only adults of this species were detected.

4.2 Seasonal dynamics

The seasonal dynamics observed in this study correspond well to dynamics described in the literature (Becker et al., 2010; Schaffner et al., 2001). The first species in the season are the so-called snow-melt mosquitoes like *Ae. cantans, Ae. annulipes, Ae. rusticus* and *Cs. morsitans*. Larvae of these species occur in breeding sites readily available in the nature reserve such as forest or forest edge pools (preferably with leaf litter), open permanent or semi-permanent meadow pools or diverse open or shaded water bodies such as ditches and small ponds (Becker et al. 2010; Schaffner et al. 2001). They were observed at the start of the monitoring in April. Eggs of *Ae. cantans* and *Ae. annulipes* are hibernating and hatch as soon as the breeding site temperature reaches 6-8°C (Schaffner et al., 2001), while a first larval population of *Ae. rusticus* and *Cs. morsitans* hatches in autumn and overwinters in the larval stage, surviving even under a cover of ice (Becker et al., 2010). A second population might hatch in spring. As observed in our study, larvae of *Ae. rusticus* and *Cs. morsitans* can often be found together in the same breeding site in spring, as they both need deeper depressions that do not freeze completely in winter. Adults of *Ae. cantans* and *Ae. annulipes*, two ecologically similar species, were caught from May on up to August with a strong peak in June, while *Ae. rusticus* were detected only in May and June. *Cs. morsitans* adults were also expected to be caught early in the season, which was not the case. But their catch result might also be influenced by the fact that they prefer birds as their blood host (Becker et al., 2010) and are not easily entering the CDC traps. They can be observed until late in the season (Becker et al., 2010), in our case up to September.

Another “type” of mosquito species are the flood-water species *Ae. vexans* and *Ae. sticticus.* They are summer species - Ae*. vexans’* optimal development temperature is 30°C (Becker et al., 2010), - and their larvae appear after flooding in springtime when the snow-melt mosquitoes have already hatched. Water level data from the Lac de Neuchâtel shows two rises in mid-May and mid-June due to heavy precipitation (BAFU, 2019). At this point in the season the lake level can cause floodings of the wetlands which might have resulted in mass hatching of these species and which would explain the peaks in adult catches in June. The vast majority of the two species was caught at site 15 (Chablais de Cudrefin) in June - a special area of alluvial forest which is artificially flooded in spring and early summer (this year from end of April till the end of June) when the local stream used for water supply is running water. Our catch numbers show that this flooding regime fits the hatching process of *Ae. vexans* and *Ae. sticticus*. Furthermore, June was an extraordinary warm month in 2019 (MeteoSchweiz, 2019). However, the hatching is not reflected in the number of larvae caught in May or June. This might be explained by the fact that water temperatures of the larval breeding sites are not necessarily depended on the lake water temperature and can vary locally (pers. communication A. Gander). High water temperatures lead to a fast development from hatching to emergence of adults (at 15°C it is 3 weeks, at 30°C 1 week (Becker et al., 2010)). In this way the presence of larvae could have been missed in the monthly monitoring rounds.

For other commonly found species such as *Ae. cinereus-geminus* or *Cx. pipiens-torrentium,* typical seasonal dynamics were observed (Becker et al., 2010): The larvae appear in late-spring, the adults are present throughout the summer months with peaks in June or July. They were observed until the end of the monitoring end of September and can usually be found up to the first frost (Schaffner et al., 2001).

4.3 Mosquito species in urban, peri-urban and nature zone

The sites in this study were chosen to represent urban, peri-urban and nature environments. Most mosquito species have their preferred breeding site in wetlands such as the “Grande Cariçaie” where resting sites and food are available. As soon as a female has emerged and mated, it starts to look for a blood host. The blood is needed for the development of the eggs. Some species stay near their breeding sites and will not fly over open land. But certain species are able to migrate over longer distances if population pressure is high. So they might move to peri-urban or urban areas in order to find a blood meal (Becker et al., 2010).

In nature sites, the average adult catches per site were higher than in peri-urban areas, which meets our expectation, as females looking for a blood meal will readily respond to the CO_2 in the CDC traps positioned near their breeding site. Average larval catches were slightly higher in peri-urban areas than in the nature zone, but this can be explained by the two extraordinarily productive sites 5 and 7 where the majority of larvae was caught. Also, possibilities for dipping and accessibility varied across the sites and might have influenced the result.

However, as already observed when choosing sites, the three zones urban, peri-urban and nature do not differ much in their characteristics. Most of the territory is rather rural and as the nature reserve is split up into different parts, natural mosquito breeding sites are never far away. Only one urban site could be defined (and is therefore not treated as an extra category in the discussion) and also some of the peri-urban sites are rather similar to a nature site. This also shows when looking at the variability in catches per site – ranging from 11 to 1'306 adults. The attractiveness of the individual sites was more important than the zone and varied greatly.

Consequently, it is not surprising that some of the most abundantly found species are the same in both nature and peri-urban zone. The nature zone is dominated by the common native wetland or floodwater mosquitoes *Ae. cantans-annulipes, Ae. sticticus, Ae. vexans,* and *Ae. cinereus-geminus* (in order of quantities caught). In the peri-urban zone *Ae. cantans-annulipes* and *Ae. cinereus-geminus* were also the two most abundantly found species, followed by *Cx. pipiens-torrentium,* and *Ae. rusticus*.

Some differences between nature and peri-urban zones still arise. Quantities cannot be compared directly, as the number of sites was not the same in the different zones. Therefore species are compared by ranking them in order of quantities caught. *Ae. vexans* and *Ae. sticticus* which were among the four most abundant species in nature sites, are only on position five and six in peri-urban sites. If the population pressure is high, these species migrate over long distances to find blood meals (Becker et al., 2010), but this was not observed in our case.

Furthermore, we found some species that were caught predominantely in either nature or peri-urban areas. The species *An. claviger-petragni,* and *Cs. morsitans* were almost exclusively caught in the nature zone. For *An. claviger-petragni* this is only true for adults (a majority of them was recorded in nature site 8). Their larvae were detected more often in peri-urban areas than in nature sites. Becker et al., 2010 says that these larvae are very sensitive to disturbance and dive quickly. So a possible explanation is that they are simply easier to catch in artificial breeding sites in a peri-urban environment than in nature sites. *Cs. morsitans* is a typical "nature" species mainly feeding on birds, so they are probably not interested in moving towards settlements.

Other species were caught more often in peri- urban areas than in the nature zone. These are adults of *Cx. pipiens-torrentium, An. plumbeus, An. maculipennis s.l.* and *Cs. annulata.* All of them feed on mammals, some populations even show a preference for humans. They all use a variety of habitats and breeding sites – including artificial containers (Becker et al., 2010), which they can easily find in a non-nature environment. We did not find larvae of all of these species in the peri-urban zone, but breeding sites can be hard to find, especially if the species is not abundant.

Cs. annulata is an interesting case: it was commonly found as larvae in peri-urban sites (majority in site 5), but only rarely as adult (only 4 in total) and exclusively in the peri- urban sites. It is said to respond well to CO_2 baited traps (Petric 1989 in Becker et al., 2010). It could be that the population in the nature reserve and its surrounding prefers birds (Schaffner et al., 2001) and is therefore less attracted to CO_2. Another explanation could be that the height of the trap was not suitable for this population.

4.4 Interaction of mosquito fauna from nature reserve with peri-urban areas

One goal of this study was to investigate if the mosquito fauna from the nature reserve can induce nuisance on surrounding settlements. Our results show first of all that all of the species found abundantly in peri-urban areas can be aggressive biters (Becker et al., 2010; Schäfer et al. 1996, Schaffner et al., 2001).

However, in the case of the “Grande Cariçaie” the geographical and landscape difference between the nature and non-nature zones is not so distinct and it is difficult to draw the line. Usually an interaction of mosquitoes from a nature area with surrounding settlements can be determined if typical “nature” mosquito species that have a big enough migration range are also found in residential areas. In the “Grande Cariçaie” mosquitoes do not necessarily have to make a big distance from their habitat or breeding site to find a blood meal in one of the local settlements. *Ae. cantans-annulipes*, for example do not fly far away from their breeding sites, but are at least able to cross open spaces, while for *Ae. cinereus-geminus* there are some contradictory statements in the literature regarding the migration range (Becker et al., 2010; Schäfer et al. 1996; Schaffner et al., 2001). *Ae. rusticus* does not fly long distances at all (Becker et al., 2010; Schäfer et al. 1996; Schaffner et al., 2001). This suggests that these species have their breeding sites near to the peri-urban sites where they were caught and do not migrate from the nature reserve itself.

Another abundant adult species in peri-urban areas was *Cx. pipiens-torrentium*. The biotype usually found in urban surroundings is *Cx. pipiens* form *modestus* and it is the most common native container-breeder, which can be found in nearly every kind of water source (Becker et al., 2010, Schaffner et al., 2001). Their high catch number is therefore more probably due to specimens that develop directly in the peri-urban zone. If residents complain about mosquitoes on their property, all pots and other containers with standing water should be emptied to reduce the population (Flacio et al., 2015; Swiss Mosquito Network, 2019).

Summing up, the “Grande Cariçaie” shows a strong connection and exchange between nature and non-nature zones for mosquitoes. Therefore, it is hard to detect an influence of one zone on the others. Furthermore, none of these species in peri-urban areas was caught in quantities that can actually cause nuisance for residents. In a year like 2019 with standard weather and lake level conditions, breeding mosquitoes of the nature reserves are not a major nuisance for residents.

5 Conclusion

In the first extensive study on *Culicidae* in the nature reserve “Grande Cariçaie” we found a rich diversity of 17 different native species which represents almost half of all reported mosquito species of Switzerland and emphasizes the good state of biodiversity in the nature reserves.

We investigated a previously unstudied wetland and covered the period from April to September 2019 with a monthly monitoring of female adult mosquitoes with CDC traps as well as with monthly dipping of larvae.

A total of 3’313 females and 252 larvae could be identified to the species level. The species composition in the nature sites is representative for a nature reserve: The snow-melt mosquitoes *Ae. cantans-annulipes* dominate both in nature and non-nature zones. Other commonly caught species in the nature zone were the flood-water mosquitoes *Ae. vexans* and *Ae. sticticus* as well as *Ae. cinereus-geminus*. The total catch numbers are comparatively low, as in other studied wetland areas the Culicidae fauna is much more dominated by flood-water mosquitoes. The “Grande Cariçaie” does not have such huge floodplains, so the proportion of these species is not as high.

Cx. pipiens-torrentium and *Ae. rusticus* were the two most abundant species in peri-urban sites next to the above mentioned *Ae. cantans-annulipes* and *Ae. cinereus-geminus*. On average more adults per site were detected in the nature zone, but a few species were also more commonly caught in peri-urban areas than in the nature zone. These were *Cx. pipiens-torrentium, An. plumbeus, An. maculipennis s.l.* and *Cs. annulata*, which all also feed on humans and use artificial containers for breeding.

The quantities caught in 2019 do not indicate that residents were disturbed by mosquitoes. Interaction of the Culicidae fauna of the nature reserve with surrounding settlements is difficult to determine as in this study the characteristics of the nature and non-nature sites were rather similar. All of the abundantly found species may as well breed near to the peri-urban site where they were caught. This is especially likely for the abundantly found container-breeder *Cx. pipiens-torrentium*. Females emerging from artificial breeding sites like garden pots, vases or rain barrels could just as well be a source of nuisance. Removing all standing water in such containers is recommended.

Our results contribute to gain a better overview of the Swiss mosquito fauna, which is becoming even more important with the recent re-emergence of mosquito-borne viruses in Europe. Judging from the catch numbers and the lack of complaints about nuisance, there is no need for a close mosquito monitoring, e.g. every 5 years as it is the case in other wetlands. But our study can serve as a reference to further monitor mosquito diversity biodiversity in the “Grande Cariçaie” in coming years.

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Sylvie Flämig

Bellinzona, 28.01.2020

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Annex

A 1 Descriptions and coordinates of sampling sites (in French)

No. site	Description	LV03_E	LV03_N	urban / periurban / nature	General environment
1	jardin urbain dans ville	538976	180804	urb	ville
2	lisière forestière, prairie marécageuse peu inondée	540251	181508	periurb	interface zone urbaine industrielle - marais boisé et non boisé
3	cordon forestier alluvial, aulnaie noire	543091	183017	nat	marais boisé et non boisé
4	jardin dans village	546682	182936	periurb	jardin urbain, cours d'eau, forêts, plans d'eau
5 (avr-juin)	loupes inondables sous souches renversées, saulaie alluviale	546927	183806	periurb	interface village - marais boisé et non boisé
5 (juil_sept)	aulnaie noire	547095	183751	periurb	marais boisé
6	cordon forestier alluvial, saulaie buissonnante	548503	184001	nat	marais boisé et non boisé
7	cordon forestier alluvial, saulaie buissonnante	550265	185773	periurb	interface village de vacances - marais boisé et non boisé
8	aulnaie noire alluviale	551941	186889	nat	marais boisé et non boisé
9	cordon forestier alluvial, saulaie buissonnante	554855	189363	periurb	interface zone urbaine de loisir - marais t non boisé
10	forêt alluviale, ripisylve mixte	558465	192942	nat	marais boisé (non boisé)
11	forêt alluviale, ripisylve mixte	559905	193868	nat	marais boisé et non boisé
12	arbre isolé, prairie marécageuse, Cladietum, Caricetum elatae	562129	195771	nat	marais non boisé
13	forêt alluviale, ripisylve mixte	563294	196821	periurb	interface village de vacances - marais boisé et non boisé
14	lisière de Pinède alluviale	565948	199106	nat	marais boisé et non boisé
15	cordon forestier alluvial, saulaie buissonnante	569897	202604	nat	marais boisé et non boisé (présence de vaches)

A 2 Full list of species

Species	Found as	
	Larva	Adult
*Anopheles (Anopheles) claviger (*Meigen 1804) - *Anopheles (Anopheles) petragnani* Del Vecchio 1939	x	x
Anopheles (Anopheles) maculipennis s.l. Meigen 1818	x	x
Anopheles (Anopheles) plumbeus Stephens 1828	x	x
Aedes (Aedes) cinereus Meigen 1818 - *Aedes (Aedes) geminus* Peus 1970	x	x
Aedes (Aedimorphus) vexans vexans (Meigen 1830)	x	x
Aedes (Finlaya) geniculatus (Olivier 1791)	x	x
Aedes (Ochlerotatus) cantans (Meigen 1818) - *Aedes (Ochlerotatus) annulipes* (Meigen 1830)	x	x
Aedes (Ochlerotatus) flavescens Müller 1764	x	
Aedes (Ochlerotatus) sticticus Meigen 1838		x
Aedes (Rusticoidus) rusticus Rossi 1790	x	x
Coquillettidia (Coquillettidia) richiardii (Ficalbi 1889*)*		x
Culex (Culex) pipiens Linnaeus 1758 */ Culex pipiens pipiens* biotype *molestus* Forskal 1775 - *Culex (Culex) torrentium* Martini 1925	x	x
Culex (Maillotia) hortensis hortensis Ficalbi 1890	x	
Culex (Neoculex) territans Walker 1856	x	
Culiseta (Allotheobaldia) longiareolata (Macquart 1838)	x	
Culiseta (Culicella) morsitans (Theobald 1901)	x	x
Culiseta (Culiseta) annulata Schrank 1776	x	x

* 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 report, the traditional names are used, i.e. *Aedes* is considered as the genus, and *Ochlerotatus* and *Rusticoidus* as subgenera for the *Ochlerotatus* spp. sensu Reinert (2000), the same with *Aedes (Finlaya) geniculatus* for *Ochlerotatus (Finlaya) geniculatus* sensu Reinert (2000).

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Reinert, J.F., Harbach, R.E., Kitching, I.J. (2004). Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zoological Journal of the Linnean Society,* 142: 289–368.

A 3 Full seasonal tables for adults and larvae

Adults

Site/Month		*Ae. cant.-annulip.*						*Ae. cinereus-gem.*						*Ae.geniculatus*						*Ae.rusticus*					
		4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
U	1	-	-	109	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	1-	22	-	-	-
PERI-URBAN	2	-	-	4	9	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
	5	-	4	11	5	-	-	-	-	-	5	2	-	-	-	-	-	2	-	-	1	3	-	-	-
	7	-	28	22	11	2	-	-	1	44	15	-	2	-	-	5	-	-	-	-	18	14	-	-	-
	9	-	-	6	9	1	-	-	2	20	24	6	-	-	-	-	-	-	-	-	13	-	-	-	-
	13	-	25	12	6	-	-	-	9	1	2	2	-	-	-	-	1	9	-	-	15	-	-	-	-
NATURE	3	-	-	-	1	-	-	-	-	1	1	-	1	-	-	-	-	-	1	-	-	3	-	-	-
	6	-	-	13	7	3	-	-	-	-	19	2	6	-	-	-	-	-	-	-	2	2	-	-	-
	8	-	10	95	8	-	-	-	-	3	2	-	-	-	-	2	-	-	-	-	1	3	-	-	-
	10	-	-	12	2-	2	-	-	-	2	13	1	-	-	-	-	-	26	-	-	9	64	-	-	-
	11	-	-	179	18	2	-	-	1	22	7	3	-	-	-	1	-	-	-	-	4	4	-	-	-
	12	-	-	4	-	-	-	-	-	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	14	-	1	102	1	1	-	-	-	262	10	4	-	-	-	7	-	-	-	-	2	9	-	-	-
	15	-	1	158	25	-	-	-	1	29	16	2	-	-	-	1	16	-	-	-	3	13	-	-	1
ALL SITES		-	69	727	12-	11	-	-	14	392	114	23	11	-	-	16	18	37	1	-	79	137	-	-	1

Site/Month		*Ae.sticticus*						*Ae. vexans vexans*						*An. claviger-petrag.*					
		4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
U	1	-	-	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
PERI-URBAN	2	-	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	1	-	-	-	-	-	5	-	-	-	-	-	-	-	-
	7	-	-	13	5	4	-	-	-	13	18	6	1	-	1	-	-	-	-
	9	-	-	-	1	1	-	-	-	2	16	1	-	-	-	-	-	-	-
	13	-	-	-	3	-	-	-	-	-	1	8	1	-	-	-	-	-	-
NATURE	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	1	1	2	-	-	-	-	2	3	-	-	-	-	-	-	1
	8	-	-	3	2	-	-	-	-	-	4	1	-	1	1	2	1	-	6
	10	-	-	-	9	3	-	-	-	-	7	11	-	-	-	-	1	-	-
	11	-	-	2	3	3	-	-	-	5	4	7	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	14	-	-	34	-	2	-	-	-	28	12	23	1	-	-	2	-	-	-
	15	-	-	381	145	1	-	-	1	356	62	25	1	-	-	-	-	-	-
ALL SITES		-	-	437	172	16	-	-	1	405	131	86	4	1	2	4	3	-	7

Site/Month		*An. maculip. s.l.*						*An. plumbeus*						*Cq. richiardii*					
		4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
U	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PERI-URBAN	2	-	-	-	-	-	-	-	1	-	-	-	4	-	-	-	1	-	-
	4	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	5	-	-	-	-	-	-	-	-	-	1	-	5	-	-	-	2	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	-
	9	-	-	-	4	2	-	-	-	-	-	-	-	-	-	-	8	-	-
	13	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-
NATURE	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
	6	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	2	-
	10	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-
	11	-	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-
ALL SITES		-	1	-	5	3	-	-	1	5	1	1	11	-	-	-	33	3	-

Site/Month		*Cx. pipiens-torr.*						*Cs. annulata*						*Cs. morsitans*					
		4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
U	1	2	-	-	82	-	1	-	-	1	-	-	-	-	-	-	-	-	-
PERI-URBAN	2	1	-	-	4	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	1	-	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-
	7	1	1	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	1
	9	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2
	13	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	4
NATURE	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2
	10	-	-	-	2	3	-	-	-	-	-	-	-	-	-	-	-	-	12
	11	-	-	6	-	3	-	-	-	-	-	-	-	-	-	-	-	-	9
	12	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	2	1
	14	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	8	15
	15	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	1
ALL SITES		5	2	7	92	26	5	-	-	3	-	-	1	-	1	-	-	16	52

Larvae

		Ae. cant.-annulip.						*Ae. cinereus-gem.*						*Ae. flavescens*						*Ae. geniculatus*						*Ae. rusticus*						*Ae. vexans vexans*						*An. claviger-petrag.*						*An. maculip. s.l.*					
Site/Month		4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
U	1	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PERI-URBAN	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
	5	22	4	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-
	7	14	3	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	13	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	6	8	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	11	2	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	12	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-
	14	7	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	1	-	-	5	-	-	-	-	-	-	-	-	-	-	-	2	-
	15	6	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ALL SITES		85	11	-	-	-	-	1	15	-	-	5	-	1	-	-	-	-	-	-	1	-	-	-	-	26	-	-	-	-	-	-	2	-	-	15	-	-	-	3	-	2	-	-	-	-	1	3	-

		An. plumbeus						*Cx. hortensis hort.*						*Cx. pipiens-torr.*						*Cx. territans*						*Cs. annulata*						*Cs. longiareolata*						*Cs. morsitans*					
Site/Month		4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9	4	5	6	7	8	9
U	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	1	-	-	-	-	-
PERI-URBAN	2	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2	-	2	6	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NATURE	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	12	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-
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ALL SITES		1	-	-	-	1	-	-	1	-	-	-	-	-	10	-	-	1	4	-	2	-	-	4	1	-	7	2	-	2	6	-	-	-	-	-	6	11	13	-	-	-	-