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Inventory of Culicidae in and around the Grande Cariçaie Nature Reserves at Lake Neuchâtel, Western Switzerland

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Abstract

For the first time an extensive study on mosquitoes (Diptera: Culicidae) was carried out in and around the Grande Cariçaie nature reserves, a wetland area along Lake Neuchâtel in Western Switzerland. This study contributes to improve knowledge on Swiss regional mosquito distribution and diversity, which is important both from a conservation perspective and for a possible assessment of future risks for human and animal health. Fifteen sites were sampled monthly between April and September 2019 by setting CDC miniature light traps and by investigating larval habitats. The sampling sites were located either in a natural, peri-urban or urban environment. Overall, a total of 3,674 mosquito specimens were identified. A high species diversity with 17 different species and a species composition typical for a wetland area with *Ae. annulipes-cantans*, *Ae. vexans*, *Ae. sticticus*, *Ae. cinereus-geminus* and *Ae. rusticus* as dominant species, was found. A strong overlap between the three categories of environments regarding species abundance, richness and composition was observed. No invasive mosquito species were recorded in the study area.

Key Words

Biodiversity, monitoring, mosquito fauna, wetland

Introduction

Culicidae (Diptera, Nematocera) are major vectors for the transmission of disease pathogens in many parts of the world. In Switzerland, after the eradication of endemic malaria in the 1930s, public interest in the study of mosquitoes remained limited. However, in recent years, public perception has shifted due to the spread of invasive mosquito species in Switzerland (Schaffner et al. 2009; Medlock et al. 2012; Suter et al. 2015; ECDC 2024a), multiple outbreaks of mosquito-borne diseases in neighbouring countries (Succo et al. 2016; Venturi et al. 2017; Barzon 2018; Bordi et al. 2018; Lindh et al. 2019; ECDC 2024b) and, more recently, the first detection of West Nile virus in mosquitoes in Switzerland in 2022 (Cazzin et al. 2023). Consequently, the need to enhance our understanding of mosquito distribution and diversity across different regions of Switzerland has become increasingly urgent.

Currently, 41 mosquito species have been recorded in Switzerland (Schaffner 2024). However, with the exception of southern Switzerland, where mosquito fauna has been studied extensively (Focarile 1987; Fouque et al. 1991; Flacio et al. 2014; Wagner et al. 2018; Flacio and Flämig 2019a, 2019b), knowledge of mosquito populations in other regions of Switzerland remains scarce or outdated (Raboud 1980; Schaffner and Mathis 2013).

One such understudied region is the nature reserves of the Grande Cariçaie in western Switzerland, where little was previously known about the faunistic status of Culicidae. The official database of the nature reserve organization contains only sporadic records of *Aedes communis* (De Geer 1776) and *Culex pipiens* (Linnaeus, 1758) from the 1980s. This study presents the first comprehensive documentation of Culicidae in the Grande Cariçaie nature reserves, providing valuable insights for both conservation efforts and the assessment of potential future risks to human and animal health.

Materials and methods

Sampling location

The Grande Cariçaie nature reserves in Switzerland form a 2,300-hectare wetland along the southern and eastern shores of Lake Neuchâtel, a foreland lake of the Jura mountains, at an altitude of 430 m a.s.l. Comprising eight distinct nature reserves spreading over 40 km of lakeshore, it represents the largest wetland area in Switzerland and is protected under the Ramsar Convention. The city of Yverdon-les-Bains, with a population of 30,202, is situated at the southern border of the study area. The landscape surrounding the nature reserves consists primarily of small settlements and agricultural land.

Sampling sites

A total of 15 sampling sites were investigated (Fig. 1), of which 13 were located within the nature reserves and 2 outside. Among these 15 sites, one site was situated in an urban environment within the city of Yverdon-les-Bains, six were positioned near residential zones ("peri-urban sites"), and eight were located in entirely natural environments ("nature sites").

Sampling methods and sampling period

Mosquitoes were sampled monthly from April to September 2019, resulting in six collections during the study period. Adult mosquitoes were captured using CDC miniature light traps (Model 512, John W. Hock Company, Florida, USA) baited with CO_2 (dry ice pellets) as an attractant. These traps target blood-seeking female mosquitoes. Traps were hung up approximately 1.5 m above the ground, and mosquitoes were collected after 24 hours. Specimens were then euthanised by exposure to dry ice and stored at -20 °C.

Larval sampling was conducted monthly during the same period by inspecting potential larval habitats. All accessible standing water bodies, such as ponds or ditches near the adult trapping sites, were surveyed. Generally, the same water bodies in a similar radius around each sampling site were examined in each collection, though some temporary water bodies dried up or became inaccessible. A standard pint dipper (Model 1132H, BioQuip Products, USA) was used, with water collection repeated three times per sampling site.

To also detect invasive mosquito species that breed in urban environments, additional larval sampling was conducted in July and August, the peak season for these species. At (peri-)urban sites, containers such as garden pots and birdbaths were inspected, if present at the site. Water from these containers was emptied into a white plastic tray, and larvae were collected using a plastic pipette. All collected larvae were preserved in 70% ethanol for storage and further analysis.

Identification

Adult and larval mosquitoes were identified to the species or species group level using morphological keys for female mosquitoes and larvae (Romi et al. 1997; Schaffner et al. 2001; Becker et al. 2010; Gunay et al. 2018). In several cases, species were grouped due to the difficulty or impossibility of differentiation based on morphological characteristics alone. Molecular methods were not applied in this study. The following species groups were considered:

- *Anopheles claviger-petragnani*: *Anopheles claviger* (Meigen, 1804) and *Anopheles petragnani* (Del Vecchio, 1939). The latter has not yet been detected in Switzerland (Schaffner 2024).
- *Culex pipiens-torrentium*: *Culex pipiens* form *pipiens* (Linnaeus, 1758) and *Culex pipiens* form *molestus* (Ficalbi, 1890), as well as *Culex torrentium* (Martini, 1925).
- *Aedes cinereus-geminus*: *Aedes cinereus* (Meigen, 1818) and *Aedes geminus* (Peus, 1970).
- *Aedes annulipes-cantans*: *Aedes cantans* (Meigen, 1818) and *Aedes annulipes* (Meigen, 1830).

Additionally, *Anopheles maculipennis* (Meigen, 1818) was referred to as *An. maculipennis s.l.*

Only adult females were included in the analysis. For larvae, only third and fourth instar stages were considered, as smaller larvae are difficult to identify using the available morphological keys, which are designed for more developed larval stages.

Calculation of the Shannon–Wiener Index

Species diversity was quantified using the Shannon–Wiener index (H′), which accounts for both species richness and evenness in the community. The index was calculated as $H' = -\sum p_i \ln(p_i)$, where p_i is the proportion of individuals belonging to species i in the sample ($p_i = n_i / N$). Higher values of H′ indicate greater diversity and a more even distribution of individuals among species.

Results

Taxonomic diversity of collected mosquitoes

A total of 17 Culicidae species belonging to five genera were recorded: *Aedes* (7 species), *Anopheles* (3), *Coquillettidia*

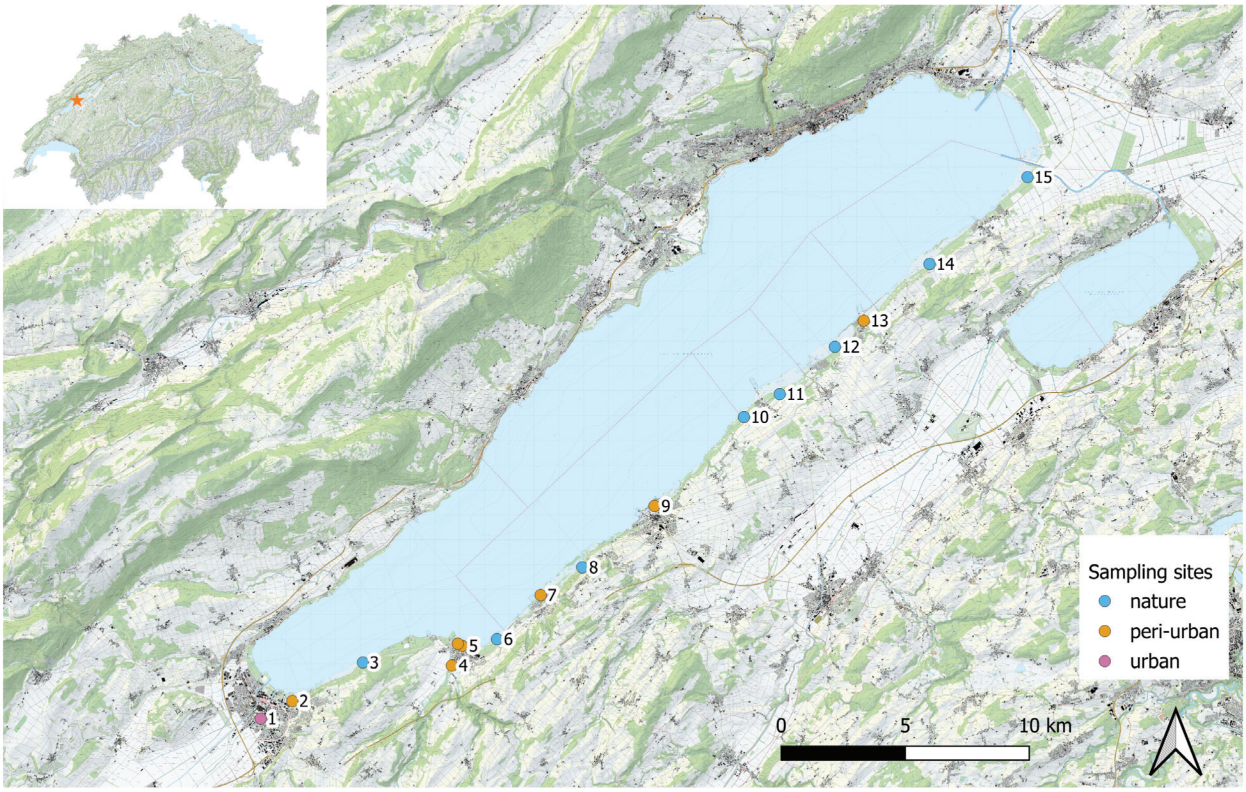

Figure 1. Map of study sites (N = 15) in the Grande Cariçaie nature reserves and its surroundings. A violet dot represents a study site in an urban environment, orange are study sites in a peri-urban environment and blue are in a natural environment. Background picture obtained from the Swiss Federal Office of Topography swisstopo.

(1), *Culex* (3), and *Culiseta* (3) Most species occurred in at least the natural and peri-urban habitats, or across all three habitat types, with one species restricted to natural sites and two exclusive to the urban site (Fig. 2). The highest number of species was recorded in peri-urban sites (13 species), followed by natural sites (12) and the urban site (7) (Suppl. material 1: table S1). The pattern was similar for larvae: 10 species were recorded in both natural and peri-urban habitats, whereas 6 species were found in the urban site (Suppl. material 1: table S2).

Mosquito abundance in nature and (peri)-urban areas

Across six sampling rounds from April to September 2019, a total of 3,674 mosquitoes were collected from the 15 study sites, comprising 3,422 adult females captured in CDC traps and 252 larvae collected through larval sampling (Figs 3, 4). Additionally, 66 adult males were counted, which had entered the CDC traps (data not analysed). The number of adult female mosquitoes collected per site ranged from 11 to 1,306 individuals, while larval numbers varied between 3 and 50 specimens per site (Suppl. material 1: tables S1, S2). Among the adult mosquitoes, five species accounted for 86.21% of the total catch: *Aedes annulipes-cantans*, *Aedes vexans* (Meigen, 1830), *Aedes sticticus* (Meigen, 1838), *Aedes cinereus-geminus*, and *Aedes rusticus* (Rossi, 1790). For larvae, *Aedes annulipes-cantans*, *Aedes rusticus*, *Culiseta morsitans* (Theobald, 1901), and *Aedes cinereus-geminus* were the most abundant species, together representing 66.27% of all larval specimens collected.

When comparing mosquito abundance across habitat types, adult catches per site were generally higher in nature sites than in peri-urban or urban sites, with an average of 327.25 ± 152.47 specimens per site in nature areas compared to 114.86 ± 34.94 in peri-urban and urban sites combined. In contrast, larval abundance per site was slightly higher in peri-urban sites (18.00 ± 6.72) than in nature sites (15.75 ± 1.99).

Overall, mosquito species diversity (Shannon-Wiener index) was higher in natural environments than in (peri-)urban environments (Table 1), indicating that mosquito species were more evenly represented in natural habitats.

Table 1. Mosquito species diversity in different environment categories. Shannon–Wiener diversity index (H′) values for mosquito communities across different environment categories. Urban and peri-urban sites were combined for analysis.

Environment category	Shannon–Wiener diversity index (H′)
Nature	1.637
(Peri-)urban	0.811
All environments	1.978

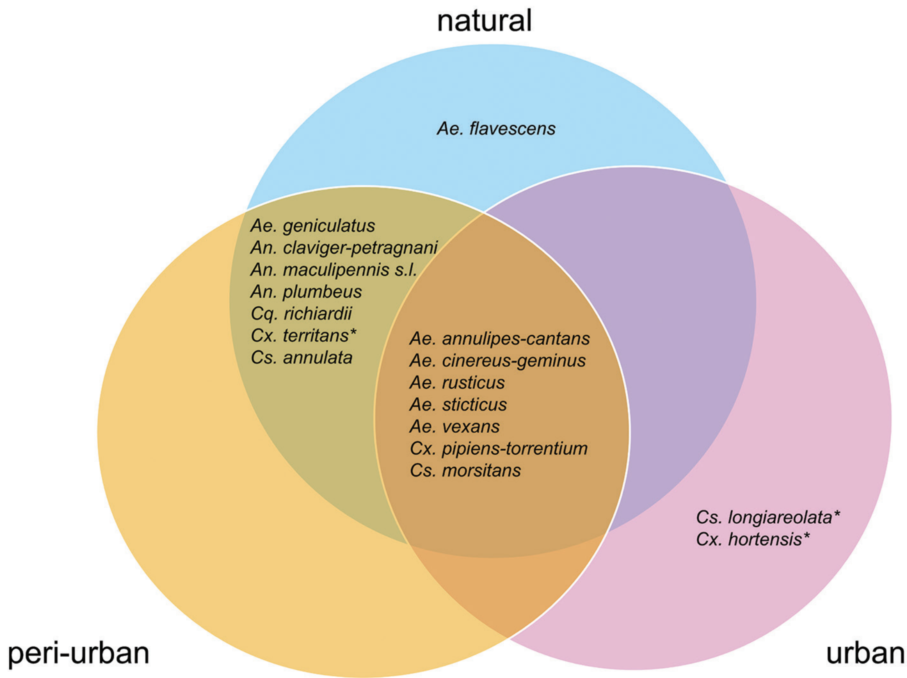

Figure 2. Venn diagram of habitats. Diagram shows the absolute presence of mosquito species found as larva or adults in natural (blue), peri-urban (orange) and urban (violet) environments. *: species was only found as larvae.

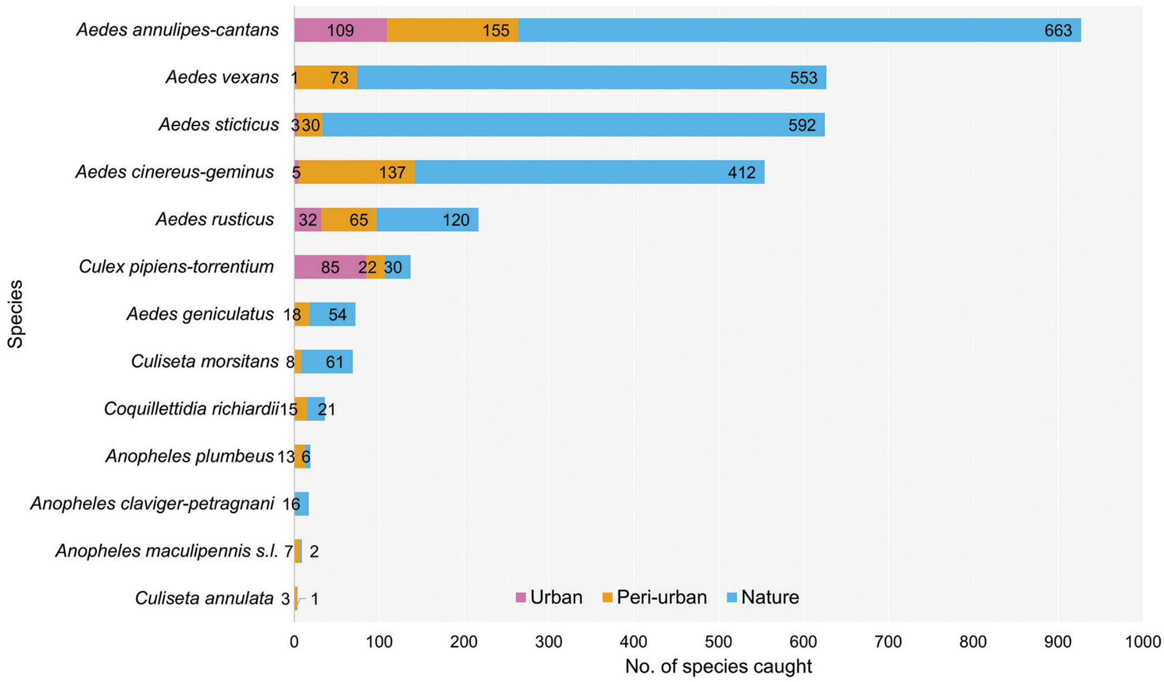

Figure 3. Number of adults caught per species and environment category. Violet represents an urban environment (n = 1), orange a peri-urban environment (n = 6) and blue a natural environment (n = 8).

Aedes annulipes-cantans was the most dominant species across all site types for both adults and larvae. Adults of this species were most abundant in the urban site, comprising 46.19% of all mosquitoes collected there, while its larvae were most frequently found in peri-urban sites, making up 45.79% of the larval collection. The floodwater species *Aedes vexans* and *Aedes sticticus* were proportionally most abundant in nature sites, representing 21.12% and 22.61% of the adult population, respectively. Meanwhile, *Aedes cinereus-geminus* was dominant in adult stage in peri-urban sites, accounting for 24.12% of the total catch. Among larvae, *Aedes rusticus* (15.87%), *Culiseta morsitans* (14.29%), and *Aedes cinereus-geminus* (11.11%) were proportionally most common in nature sites (Figs 3, 4).

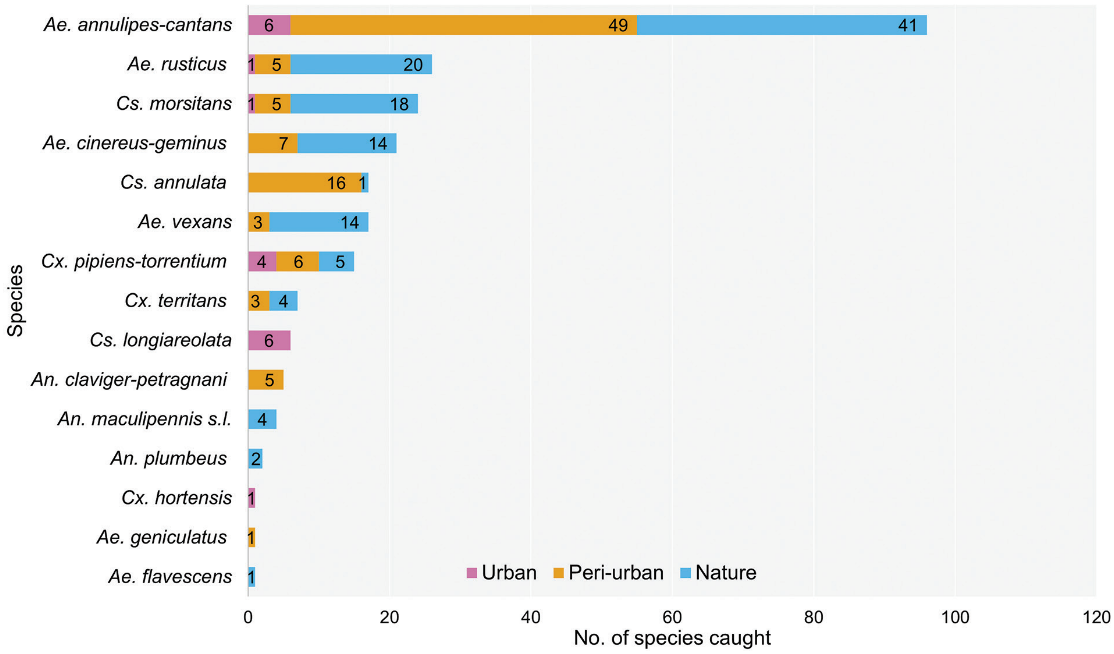

Figure 4. Number of larvae caught per species and environment category. Violet represents an urban environment (n = 1), orange a peri-urban environment (n = 6) and blue a natural environment (n = 8).

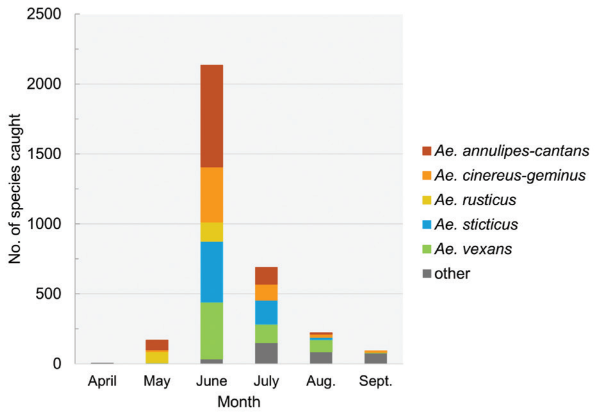

Figure 5. Adult catches per monthly catching round (April – September). The five most abundant species are shown separately, the remaining species are grouped into "other".

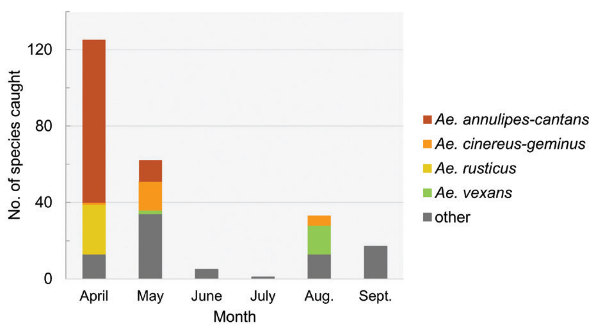

Figure 6. Larval catches per monthly catching round (April – September). The four most abundant species are shown separately, the remaining species are grouped into "other".

Seasonal dynamics

The seasonal dynamics of mosquito populations showed clear trends over the study period. In April, adult mosquito abundance was still very low, while larval numbers peaked, dominated by *Aedes annulipes-cantans* and *Aedes rusticus* (Figs 5, 6). The highest abundance of adult mosquitoes was observed in mid-June, with more than three times as many adults collected compared to July, which had the second-highest adult population.

Among the five most commonly recorded species, *Aedes rusticus* and *Aedes annulipes-cantans* were already present in May, whereas *Aedes vexans* and *Aedes sticticus*, which require flooded habitats for development, only appeared in mid-June. Their emergence coincided with two episodes of rising water levels in Lac de Neuchâtel in mid-May and mid-June, likely triggered by heavy precipitation (FOEN 2023). *Aedes cinereus-geminus* larvae were most frequently collected in mid-May, with adults peaking in mid-June and mid-July. Less abundant species generally peaked later in the season, with adults reaching their highest numbers in July and larvae peaking in May.

Discussion

Species diversity and relative abundance

This study is the first comprehensive investigation of Culicidae diversity both in the Grande Cariçaie and in Western Switzerland north of the Alps. In an alpine country like Switzerland, it is particularly important to examine mosquito diversity across different regions. The Alps act as natural barriers, influencing climatic conditions, seasonal dynamics, and habitat characteristics. Consequently, knowledge gained from mosquito populations south of the Alps cannot be directly applied to other parts of the country.

While mosquitoes inhabit a wide range of environments – with some cold-adapted species even occurring at higher altitudes in alpine regions – wetlands such as the Grande Cariçaie can be considered hotspots of mosquito diversity. These habitats host a particularly rich variety of species, making them ideal sites for biodiversity studies.

The recorded total diversity of 17 mosquito species in our study represents 41% of all reported mosquito species in Switzerland (Schaffner 2024). Given the local scale of this study, this suggests a relatively high level of biodiversity in and around the Grande Cariçaie nature reserves. The taxonomic diversity observed is comparable to other studies in Swiss wetlands: Flacio et al. (2014) recorded 18 species in the Bolle di Magadino nature reserve, while Colucci et al. (2015) found 17 species in the Langholz wetland reserve.

The calculated diversity index supports these findings, despite the Shannon–Wiener index being rarely reported in studies from Switzerland or Europe. Overall diversity is comparable to one observed in an study of an Italian wetland (Möhlmann et al. 2017). Higher mosquito diversity observed in wetlands relative to (peri-)urban habitats is consistent with previous reports of greater species richness in wet and inundated natural areas (Schaffner and Mathis 2013, Flacio et al. 2014, Möhlmann et al. 2017).

Of the two mosquito species previously documented in the official database of the nature reserve association, only *Culex pipiens-torrentium* was detected in our study. *Aedes communis* was not found. In Switzerland, this species mainly occurs at higher altitudes (Schaffner and Mathis 2011), which may explain its absence in our samples. Given the limited existing records for our study area, it is not possible to determine whether *Ae. communis* could reasonably have been expected during our sampling period.

The species composition observed, with a few dominant species – *Aedes annulipes-cantans*, *Aedes vexans*, *Aedes sticticus*, and *Aedes cinereus-geminus* – alongside several rarer species, is typical for wetland areas (Schaffner and Mathis 2013, Flacio et al., 2014, Wagner et al. 2018). The high abundance of the two floodwater species, *Aedes vexans* and *Aedes sticticus*, was largely influenced by collections from a single site, Chablais de Cudrefin. This alluvial forest area is flooded in spring and early summer by a beaver (*Castor fiber*) regulated stream, creating an ideal breeding ground for both species. Their eggs, deposited in humid soil, can survive for at least five years (Becker et al. 2010). Mass hatching events occur when flooding coincides with temperatures exceeding 9 °C, often leading to significant mosquito nuisance for local residents (Becker et al. 2010; Flacio et al. 2014; Lüthy 2014). In 2019, flooding at Chablais de Cudrefin between late April and late June aligned with the hatching period of *Aedes vexans* and *Aedes sticticus*. However, no major mass hatching event was observed across the broader study area, despite rising water levels in Lake Neuchâtel in mid-May and mid-June following heavy precipitation. This suggests that mass hatching depends on multiple factors, including flood extent, duration, and timing, as well as water temperature fluctuations (Becker et al. 2010).

The fact that most species were found in both larval and adult stages confirms the effectiveness of the chosen collection methods. However, some limitations exist due to species-specific traits. *Culex hortensis* and *Culex territans* were only found as larvae, likely because these species primarily feed on reptiles, amphibians and birds (Schaffner et al. 2001) and are therefore less attracted to CO_2-baited traps. This result aligns with similar studies using the same methodology (Osório et al. 2014; Wagner et al. 2018). In future studies, other trap types could be used alongside CDC traps to reduce such effects and maximise the number of individuals caught.

The abundance of ornithophilic *Culex pipiens* form *pipiens* and its sibling species *Culex torrentium* may have been underestimated due to trap placement at approximately 1.5 meters above ground. A higher positioning might have improved catches of these bird-associated species but would have reduced captures of other species. Since the study aimed to monitor both species diversity and potential human nuisance, trap placement was optimized for anthropophilic species.

Another species that may have been more common than our larval findings suggest is *Coquillettidia richiardii* (Ficalbi, 1889). Larvae of *Coquillettidia* species cannot be sampled with dipping techniques, as they attach to aquatic plants via their siphons. Therefore, only adults of this species could be detected in the study.

Seasonal dynamics

The seasonal patterns observed in this study closely follow those described in the literature (Schaffner et al. 2001; Becker et al. 2010; Flacio et al. 2014). Early-season species, such as *Aedes annulipes-cantans*, *Aedes rusticus*, and *Culiseta morsitans*, emerged first. Their larvae develop in breeding sites readily available in nature reserves, including forest edge pools with leaf litter, open permanent or semi-permanent meadow pools, and open or shaded water bodies like ditches and small ponds (Schaffner et al. 2001; Becker et al. 2010).

The eggs of *Aedes annulipes* and *Aedes cantans* hibernate and hatch when water temperatures reach 6–8 °C (Schaffner et al. 2001). Meanwhile, *Aedes rusticus* and *Culiseta morsitans* hatch in autumn and overwinter as larvae, while a second cohort of individuals might hatch in spring (Becker et al. 2010). As indicated by this survey, these species often co-occur in the same breeding sites, particularly in deeper depressions that do not freeze completely in winter.

For other commonly recorded species, such as *Aedes cinereus-geminus* and *Culex pipiens-torrentium*, typical seasonal trends were observed (Becker et al. 2010; Flacio et al. 2014). Their larvae emerged in late spring, with adult populations peaking in June or July and persisting through September, which was the end of the study period. These species likely remained active until the first frost (Schaffner et al. 2001).

Mosquitoes in natural and (peri-)urban environments

Mosquito species exhibit different habitat preferences, breeding site choices and flight behaviours. This study aimed to explore whether mosquito diversity and abundance varied across different environment types, which could help assess whether mosquitoes from nature reserves contribute to nuisance in surrounding settlements.

Mosquito species richness in nature and peri-urban sites was similar (Fig. 2). However, as only one urban site could be included in the study, no meaningful comparison could be made for urban areas. Average adult catches per site varied widely across all categories (see Suppl. material 1: table S1), suggesting that the attractiveness of individual sites played a more significant role than habitat type.

Larval abundance was slightly higher in peri-urban areas, though this result was largely driven by two highly productive sites (sites 5 and 7). Additional factors influencing our larval catches included the supplementary sampling of human-made breeding sites (e.g. garden pots, vases) in peri-urban and urban areas during July and August, which aimed to enhance the likelihood of detecting invasive mosquitoes. Additionally, variability in access to and availability of breeding sites across locations played a role. For future studies, a more standardised approach to larval sampling should be adopted, including recording the exact type and characteristics of each breeding site, noting the absence of larvae where applicable, and ensuring better standardisation of the sampled area surrounding each site.

Some species, such as *Culiseta morsitans* and *Anopheles claviger-petragnani*, were predominantly found in nature sites, which aligns with their habitat preferences (Becker et al. 2010). Interestingly, *Anopheles claviger-petragnani* larvae were exclusively found in peri-urban sites, possibly due to easier sampling in these environments. Becker et al. (2010) states that these larvae are highly sensitive to disturbances and dive quickly. Additionally, the low sampling success of *An. claviger-petragnani* larvae could also be a contributing factor. To improve larval detection, the dipping method could be slightly adapted. Especially *Anopheles claviger* larvae often remain near the edges of their breeding sites rather than in the central areas. Therefore, sampling efforts should focus on the margins of the habitat instead of the middle to increase capture success (Becker et al. 2010).

In contrast, species such as *Culex pipiens-torrentium*, *Anopheles plumbeus*, *Anopheles maculipennis* s.l., and *Culiseta annulata* were more abundant in (peri-)urban environments. These species feed on mammals and use artificial breeding sites (Becker et al. 2010), suggesting potential movement between natural and human-altered habitats. While they are not known for travelling long distances (CDC 2024), some study sites were close enough to facilitate such migration.

A typical “nature species” that also feeds on mammals and occasionally utilizes artificial breeding sites is *Aedes geniculatus* (Becker et al. 2010). In our study, this species was more abundant as adults in natural environments. *Aedes geniculatus* primarily breeds in tree holes, which are more prevalent in natural areas. However, it is also known to occasionally exploit artificial breeding sites. Notably, eggs of this species are regularly found in oviposition traps placed in cities as part of monitoring programs (Müller et al. 2022). This suggests that *Aedes geniculatus* exhibits opportunistic behaviour when selecting breeding sites, particularly when natural options are scarce.

Similarly, *Culex pipiens-torrentium* was found to be more abundant in urban and peri-urban sites than in natural environments. These specimens might originate from populations developing directly in the peri-urban zone. However, a more precise differentiation between the species and its forms would be necessary, as they exhibit distinct habitat preferences. Furthermore, the two forms of *Culex pipiens* are known to hybridize and display behaviourally divergent ecological traits along a latitudinal gradient (Haba and McBride 2022). To better understand the behavioural and ecological differences between *Culex torrentium*, *Culex pipiens* form *molestus* and *Culex pipiens* form *pipiens*, further investigations with increased sampling effort and genetic analyses would be required.

Influence of mosquito fauna from nature reserve on surroundings

Several abundant species in peri-urban areas are known to be aggressive human biters (Schäfer et al. 1997; Schaffner et al. 2001; Becker et al. 2010). However, the interaction between nature reserve mosquitoes and nearby settlements remains unclear, as the environmental characteristics of nature and non-nature sites were relatively similar. The mosquito species observed in peri-urban areas may have either originated there or migrated from natural habitats in search of blood meals.

Absence of invasive species and potential risks from native mosquitoes

No invasive mosquito species were detected in our study. The Asian tiger mosquito *Aedes albopictus* (Skuse, 1894), an established urban pest in southern Switzerland that is increasingly found in other parts of the country (info fauna 2024a), was not found. This species is of particular concern, as it serves as a vector for at least 26 arboviruses

(Barzon, 2018). Similarly, neither the Asian bush mosquito *Aedes japonicus* (Theobald, 1901), which is widespread in Switzerland and has been detected close to our study area (info fauna, 2024b), nor the Korean mosquito *Aedes koreicus* (Edwards, 1917), currently restricted to a few regions in Switzerland (Suter et al., 2015, info fauna 2024c), were detected. Both species are regarded as having a lower relevance for the transmission of human or animal pathogens (Schaffner et al. 2013).

Nevertheless, also native mosquitoes have the potential to transmit pathogens. The recent detection of West Nile virus in *Culex pipiens* mosquitoes in southern Switzerland (Cazzin et al. 2023), together with a rising number of human infections in Europe (ECDC 2023) underscores the potential risk of local transmission. *Culex pipiens* form *pipiens* is considered the main vector for West Nile virus, while *Culex pipiens* form *molestus* functions as a bridge vector facilitating virus transmission from birds to humans or equids (Becker et al. 2010). Given that the Grande Cariçaie hosts both migratory birds and *Culex pipiens-torrentium* mosquitoes, future monitoring for West Nile virus should be considered to assess future public and veterinary health risks.

Conclusion

This study provides a valuable baseline for future mosquito monitoring in the Grande Cariçaie. It also facilitates comparisons with other regions in Switzerland and enhances our understanding of mosquito diversity north of the Alps. Given the increasing emergence of mosquito-borne viruses in Europe, continued surveillance of mosquito diversity in Switzerland remains essential.

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Supplementary material 1

Supplementary tables

Authors: Sylvie Flämig, Antoine Gander, Eleonora Flacio
Data type: docx
Explanation note: **table S1.** Number of adult mosquitoes captured per species and site; **table S2.** Number of larvae captured per species and site.

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