



Assessment of mosquito larvae (Diptera: Culicidae) productivity in urban cemeteries in Wrocław (SW Poland)

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Abstract

In many European countries, cemeteries are found to be suitable habitats for the proliferation of native and invasive mosquito species. The study aimed to assess for the first time in Poland the suitability of urban cemeteries for mosquito larvae development in water supply wells. From May to September 2019 in three urban cemeteries in Wrocław (SW Poland), a total of 757 inspection of water supply wells were carried out. The mosquito larvae development, expressed as the general container index (GCI), differed between the examined areas and months of the study. The highest GCI value was calculated for Grabiszyński and Bujwida cemeteries (72.0 % and 59.3 % respectively). Larvae of three native mosquito species were identified in all inspected breeding places during the survey: *Culex pipiens* s.l. Linnaeus (95 %), *Cx. torrentium* Martini (4 %), *Anopheles maculipennis* s.l. Meigen (less than 1 %). No larvae of invasive *Aedes* mosquito species were found. In the inspected water supply wells larval productivity peaked in June, when the average temperature reached 22.7 °C. Due to the likelihood of the establishment and adaptation of invasive aedine mosquito species in Poland, constant analysis of the larvae composition in cemeteries should be continued.

Keywords Culicidae · Larvae surveillance · Poland · Urban cemeteries

Introduction

For a better estimation of the threat of mosquito-borne diseases in European regions, studies on the occurrence of mosquito breeding sites in urban environments have been conducted (Krüger et al. 2014, Lebl et al. 2015). In addition to native mosquito fauna, surveillance of the introduction and establishment of *Aedes* invasive species has been carried out in cities, including cemeteries, of many European countries

such as in Austria (Seidel et al. 2016, Zielke et al. 2016), Croatia (Klobučar et al. 2018), the United Kingdom (Dallimore et al. 2017), France (Krebs et al. 2014), Germany (Pluskota et al. 2008, Becker et al. 2011, Schneider 2011, Werner et al. 2012, Werner and Kampen 2013, Zielke et al. 2015, Pfitzner et al. 2018), Italy (Montarsi et al. 2013, Montarsi et al. 2015), Slovenia (Kalan et al. 2017) and Switzerland (Schaffner et al. 2009). From the identified species, five of them namely *Aedes albopictus* (Skuse, 1895), *Ae. aegypti* (Linnaeus, 1762), *Ae. japonicus* (Theobald, 1901), *Ae. koreicus* (Edwards, 1917) and *Ae. atropalpus* (Coquillett, 1902) are container-breeding and anthropophilic (ECDC 2012). Their occurrence and spread constitute a serious threat to public health in Europe as most of them are vectors of numerous pathogens, in particular arboviruses such as chikungunya virus (CHIKV) and dengue virus (DENV) (Medlock et al. 2012, 2015; Kampen et al. 2016). For this reason, as well as from an anthropocentric point of view, the knowledge about suitable breeding sites for mosquito vector species such as cemeteries in urban areas is of special interest.

In almost every human settlement around the world, from megalopolises with millions of inhabitants to small villages, cemeteries are an obligatory and specified area (Vezzani et al.

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2005, Vezzani 2007). Burial sites are visited by large numbers of people but also provide a great deal of vegetation cover and a lower edification level, resulting in higher landscape connectivity. This facilitates insect breeding and dispersal. In cemeteries, there is typically also a large number of artificial containers, such as flasks, bottles, flower vases or water wells, which enable the development of preimaginal stages of mosquitoes, thus creating a diverse nuisance level of mosquito females (Vezzani 2007, Kampen et al. 2016). Cemeteries offer also appropriate habitats for adult mosquitoes such as bushes and trees that provide shade and shelter. Most cemeteries are public property making them easily accessible; this generates permanent food sources for adult mosquitoes including flowering plants left by visitors, the blood of human visitors and on-site workers, and the blood of birds and small mammals (Koban et al. 2019).

In contrast to many European and global studies, the surveys on qualitative and quantitative composition of mosquito fauna in Polish cemeteries have not been carried out and published to date. Therefore, the main objective of the present study was to estimate the breeding opportunities of Culicidae within water supply wells in the cemeteries of Wrocław (SW Poland), taking into account climate conditions, while investigating the seasonal dynamics and species composition of the immature stages of mosquitoes in cemeteries, including the presence verification of *Aedes* invasive species.

Materials and methods

Study site

The study was conducted in the southwestern Poland, in the city of Wrocław, which is the capital of Lower Silesia. Wrocław and its surrounding areas form the Wrocław Plain, which is, in turn, geomorphologically part of the Silesian Plain (Kondracki 2000). It has an elevation of 120–150 m above the sea level and is divided into two unequal parts by the wide Odra River valley. This area experiences a climate characterized by mild and short winters, early springs, and long, warm summers, which is unique for Poland (Cichoński 2006). This is a result of geographical location in Lower Silesia, within the transitory belt between the oceanic and continental influences, as well as the combined influence of the arctic-tropical and sea-continental air masses. The climate of Wrocław's region is especially marked by a rather high average annual temperature, ranging in 2019 from 0.2 °C in January to 22.7 °C in June (average 11.5 °C). Average annual precipitation is at the level of 453 mm, there is a 225-days vegetation period, and winters are endure only a short period of snow cover (<https://danepubliczne.imgw.pl>).

The study was conducted from May to September 2019 in three cemeteries namely: Grabiszyński (GR), Bujwida (BU) and Jerzmanowo (JE). Two of them (GR and BU) are located in the city center and are surrounded by houses and tall buildings (Fig. 1). The third cemetery (JE: Jerzmanowo) is located on the outskirts of the city, close (approx. 2 km) to International Airport, where there are some family homes but less tall buildings. The GR and JE cemeteries are public (run by municipal authorities) and BU belongs to the group of parish cemeteries. All the inspected cemetery areas were characterized by diverse species composition and vegetation density; however, the greatest environmental heterogeneity and landscape values occurred in GR (Gizowska and Szopińska 2013, Przewrocka and Ziemiańska 2013a,b). The current borders of the GR cemetery are one of the three parts of cemetery complex, which was founded in 1881; it has a total area of approx. 20 ha and is an object with very high natural and landscape values (Burak and Okólska 2007; <http://zck.wroc.pl/cmentarze-komunalne/oddzial-grabiszyn>). BU cemetery is a small cemetery with an area of 4.3 ha, which was founded in 1866 (Burak and Okólska 2007). JE cemetery is the smallest in the study, at 2.7 ha, and was established in 1998; the relative newness of the site means vegetation in this area is less dense than in the other studied areas (<https://www.wroclaw.pl/cmentarz-na-Jerzmanowie>).

Sampling of immature mosquitoes

Mosquito larvae were sampled from May to September 2019 within water supply wells (Fig. 2).

Water supply wells were identified in each inspected cemetery using maps obtained from the Cemeteries Board. All existing and potential breeding habitats in the study areas were recorded using a GPS receiver and transferred to the computer database. The construction of all water supply wells was similar; however, the number of wells differed across the three cemeteries: 81 water supply wells were confirmed in GR cemetery compared to only 9 in JE and 6 in BU, respectively. Additionally, the water levels in the water supply wells varied throughout the study period. All potential mosquito breeding sites were checked in cemeteries once per month in May and twice per month from June to September.

One dip was taken with a standard dipper (volume 350 ml) in each water supply well per visit. The number of larval stages L1–L2, L3–L4, and pupae were counted and recorded. All immature mosquitoes (larvae and pupae) were transferred to the laboratory where, to enable species identification, the larvae of L1–L3 were reared to L4 stage and pupae were reared to adult.

The four instar larvae (L4) were killed by thermal shock with hot water (60 °C), collected with a fine pipette, and then put in a vial filled with an 80 % solution of ethanol. The fourth instar larvae of *Culex* sp. were morphologically determined

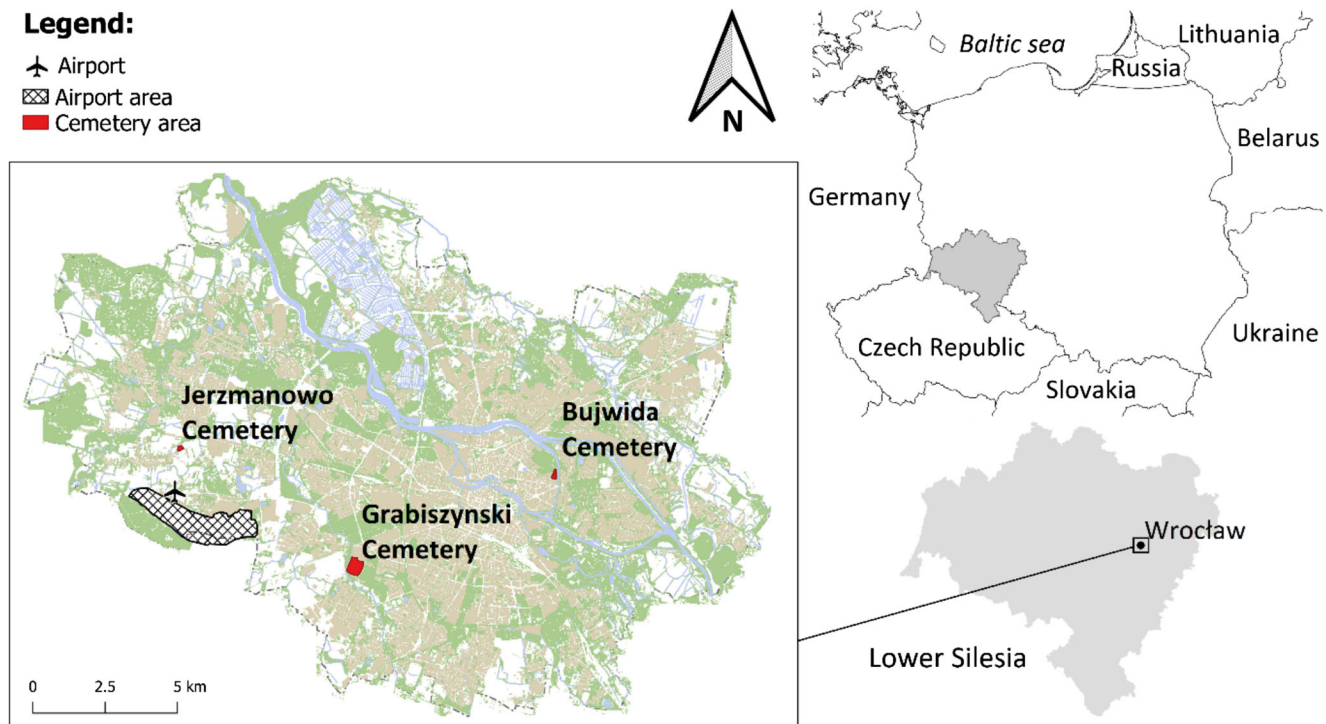


Fig. 1 Location of study sites in the Wrocław area

according to Becker et al. (2010). Using a stereoscopic microscope the number of setae 1 at the abdominal segments III to V was assessed. The larvae of *Culex pipiens* s.l. possess regularly two or two and three setae at each place, whereas *Culex torrentium* is described to have each four setae at each place (Becker et al. 2010). The larvae belonging to *Anopheles maculipennis* complex were assessed on the basis of morphological features located on the larval head including the

structure of the antennal setae, inner and outer clypeal setae as well as frontal setae (Becker et al. 2010).

Data analysis

Mosquito larvae productivity in each cemetery was expressed as the general container index (GCI) according the formula: $GCI = \text{number of breeding sites containing larvae} / \text{total number of breeding sites} \times 100$ (Petrić et al. 2014).

In the study period, meteorological data regarding average monthly precipitation, sunlight duration per day, and air temperature in Wrocław area were obtained from the Institute of Meteorology and Water Management - National Research Institute (Rocznik Meteorologiczny 2019, <https://danepubliczne.imgw.pl>).

Results

Overall, 492 (65 %) of 757 inspected water supply wells tested positive for mosquito immature stages; 448 in GR, 32 in BU and 12 in JE (Table 1). In total, larvae of three mosquito species were identified: *Culex pipiens* s.l. L. (95 %), *Cx. torrentium* Martini (4 %) and *Anopheles maculipennis* s.l. Meigen (less than 1 %). No larvae of invasive *Aedes* mosquito were found.

Mosquito larvae productivity varied between inspected cemeteries (Table 1). During the period the value of GCI



Fig. 2 An example of a water supply well widely present in selected Wrocław cemeteries

Table 1 Breeding sites measurements and container indices at all cemeteries of Wrocław

Indices	Cemetery		
	Bujwida (BU)	Grabiszyński (GR)	Jerzmanowo (JE)
Number of water supply wells	6	81	9
No. of breeding sites inspected	54	622	81
No. of breeding sites with larvae	32	448	12
General container index, % (GCI)	59.3	72.0	14.8

was the highest for GR and BU cemeteries (GCI = 72.0 % and 59.3 % respectively). In turn water supply wells in JE cemetery offered the least suitable breeding containers for mosquito larvae (GCI = 14.8 %).

The sample size in a single 350ml dip varied between 0 and 110 individuals. In the GR mosquito larvae developed throughout the whole study period, while in the BU and JE were only found from June to September (Table 2). The value of GCI varied from 0 in May (BU and JE) to 91.67 % in June and July (BU).

The most intense mosquito larvae development occurred in June and July when GCI varied from 84.25 % in the GR cemetery to 91.67 % in the BU cemetery. In August, a decrease in the development rate and number of mosquito larvae was observed in all cemeteries. This decline was also recorded in September.

Mosquito larvae productivity in the water supply wells inspected were peaked in June (GCI = 77.8 %) when the average temperature reached 22.7 °C. At that time, sunshine duration was about 12 hours and precipitation was at the lowest level (0.9 mm) of the entire study period (Table 3). On the other hand, the lowest GCI value was in May, when the average temperature reached 12.4 °C, sunshine duration was about 6 hours and precipitation was at the highest level of the study (3.2 mm).

Table 2 Mosquito larvae productivity at the cemeteries inspected from May to September 2019

Cemetery	General container index, % (Number of water supply wells inspected)				
	May	June	July	August	September
Bujwida (BU)	0 (6)	91.67 (12)	91.67 (12)	75.00 (12)	33.33 (12)
Grabiszyński (GR)	42.5 (80)	84.67 (137)	84.25 (127)	82.7 (133)	55.86 (145)
Jerzmanowo (JE)	0 (9)	16.66 (18)	11.11 (18)	22.22 (18)	16.66 (18)

Discussion

The results of our study suggest that the cemeteries of Wrocław may act as variable and important breeding areas for native mosquito species within their densely urbanized neighborhoods (Vezzani et al. 2001). The most likely explanation for the high abundance of mosquitoes and frequency of infested water supply wells during the season, is in part, due to permanent presence of water and frequent human activity in cemeteries. Our study also confirms that monitoring the breeding of mosquitoes in artificial containers is a suitable approach as these containers provide numerous habitats for larvae of native mosquito species and are easily accessible for investigators (Vezzani 2007; Kampen et al. 2016). According to Vezzani (2007), compared to pools, ponds, swamps, and rice fields, different types of artificial containers are: (a) significantly smaller and, therefore, support fewer species with smaller population sizes (b) habitats with almost no internal productivity, based mainly on the decomposition of leaf litter and other detritus, and (c) a home to larval mosquito populations, which are regulated by food limitation or competitive interactions rather than predation (Vezzani 2007). Nevertheless, cemeteries are still an underestimated breeding site for mosquitoes. Annual mosquito productivity in a cemetery is estimated to be 12 million larvae and, on any given day of the year, it can be between 2.5 and 60 million larvae (Vezzani 2007).

All the three inspected cemeteries in Wrocław differed in the area and the number of suitable breeding sites, as well as the number of mosquitoes in their immature stages. However, in the present study GR cemetery showed the highest number of water supply wells infestation and the highest GCI values. These results are similar to previous data collected in 2013 within water supply wells in another large cemetery in Wrocław – Osobowicki cemetery, when the development of mosquito larvae was observed from May to September and the GCI was 48.28 % (Rydzanicz, unpublished data). Our observations have also shown that a cemetery with a small area and a small number of wells (BU cemetery) can provide favourable conditions for mosquito development and, thus, leads to adult females causing nuisance. A survey carried out in rural and urban cemeteries in Buenos Aires showed,

Table 3 Average value of selected climatic parameters in relation to container indices at the cemeteries of Wrocław from May to September 2019

Parameter	May	June	July	August	September
Number of supply water wells inspected	95	167	157	163	175
GCI (%)	35.8	77.8	74.5	74.8	50.3
Air temperature [°C]	12.4	22.7	20.2	21.2	15.2
Precipitation [mm]	3.2	0.9	1.6	1.4	1.9
Sunshine duration per day [h]	6.6	12.1	8.3	8.2	6.6

in addition, that the mean annual temperature, photoperiod variability, shade and time of the season were key drivers of distribution of immature mosquitoes grouped in the complex *Culex pipiens* L. collected from flower vases and other artificial containers (Vezzani and Albicocco 2009, Cardo et al. 2020). Also in our study the seasonal (summer) peak of immature mosquitoes dynamics was observed mainly in June when the average temperature reached 22.7 °C and sunshine duration was about 12 hours. Given these observations, it is suggested that our future study in cemetery areas should be improved by detailed analysis on the effects of shade and vegetation on the container index and immature stages productivity of the mosquitoes.

The presence and development of *Aedes* invasive mosquito species has not been confirmed by our study. Their occurrence has been noted in neighboring countries such as the Czech Republic, Germany and Slovakia (Šebesta et al. 2012, Bocková et al. 2013, Kuhlisch et al. 2018) and on several occasions the public have been concerned about the potential presence of *Ae. albopictus* in Poland. Between 2016 and 2019, several adult mosquito females were sent to our laboratory by private persons as suspected “invasive *Aedes* species”. However, in every case they were identified as *Culiseta annulata* Schrank (80 %), *Ae. vexans* Meigen (15 %) and *Cx pipiens* s.l. in 5 % (Rydzanicz, unpublished data). Females *Cs. annulata* are known to be a biting nuisance and frequently enters people’s dwellings for hibernation.

In our study, all mosquito larvae collected from 492 positive water supply wells in Wrocław’s cemeteries were identified as *Culex pipiens* s.l., *Cx torrentium* and *An. maculipennis* s.l. but it should be emphasized that adult mosquitoes belonging to the *Culex pipiens* complex and *Anopheles maculipennis* complex cannot be reliably distinguished based on morphological characteristics only (Becker et al. 2010, Becker et al. 2012). Although, there are only 3 of the 48 species included in the Polish mosquito fauna, they are important from an epidemiological and veterinary point of view (Kubica-Biernat 1999; Rydzanicz et al. 2017). In the Holarctic *Culex pipiens* complex is known to comprise of *Cx pipiens pipiens* Linnaeus, *Cx pipiens* biotype *molestus* Forskal, *Cx quinquefasciatus* Say, *Cx pallens* Coquillett, *Cx restuans* Theobald and *Cx torrentium* Martini (considered as a separate species because of the genetical distance to *Cx pipiens*) (Becker et al. 2012). Mosquito females of *Culex pipiens* complex are recognized as the main vector of West Nile virus

(WNV) in Europe (Lebl et al. 2015). They not only cause the enzootic amplification of WNV among avian hosts, they also act as bridge vectors to mammalian hosts (Molaei et al. 2006). They have also been shown to transmit the Sindbis virus (SINV), Usutu virus (USUV) and Tahyna virus (TAHV) (Hubálek 2008). The main vertebrate hosts for TAHV are small mammals but humans can also be infected; the virus then causes an influenza-like illness (Hubálek 2008).

Furthermore, the members of *Culex pipiens* complex are important vectors in the transmission of parasitic nematodes (e.g. *Dirofilaria repens*) and protozoans which cause dirofilariasis and avian malaria (Lebl et al. 2015). In turn, *Cx torrentium* - was experimentally established as most susceptible to Sindbis Virus (SINV), an ethiological agent of Ockelbo disease (Lundström 1994).

The sibling species of *Anopheles maculipennis* complex have been shown to be infected with viruses (Batai, Sindbis, Ťahyňa, West Nile) and parasites (*D. immitis*, *D. repens*, *Setaria labiatopapillosa* and *Plasmodium vivax*) (Jöst et al. 2010, 2011; Kronefeld et al. 2012, Czajka et al. 2014, Rydzanicz et al. 2017).

Although *Culex* sp. larvae were the most abundant in the water supply wells in our study, it should be emphasized that these kinds of breeding sites are also reliable indicators of the presence of invasive *Aedes* species such *Ae. albopictus* and *Ae. japonicus*. Recent surveys carried out in the Netherlands and Germany, have shown that artificial containers in cemeteries and allotments can be suitable places for the development of many mosquito larvae including *Culex* sp., *Ae. albopictus* and *Ae. japonicus* (Ibáñez-Justicia et al. 2018; Kuhlisch et al. 2018). Given the vector potential of both native and invasive *Aedes* mosquito species as well as awareness of the introduction of invasive species in combination with climatic and environmental changes, may lead to an increase likelihood of the establishment, adaptation and developmental rate of vectors to new environments. With this in mind, the search for suitable mosquito breeding sites in Polish cemeteries should be continued and expanded.

Conclusions

In the city of Wrocław, cemeteries are suitable areas for the breeding of native mosquito species. The development rate of mosquito larvae in the cemeteries is constant, which results in

females causing a nuisance to both guests and local residents. The developmental rate of mosquito larvae of native species within generally accessible water supply wells is dynamic and adapted to the current environmental conditions. For this reason and due to the likelihood of the establishment and adaptation of new invasive vector aedine species in Poland, constant analysis of the quantitative and qualitative composition of mosquito larvae in cemeteries should be continued.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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