

## Distribution and ecology of mosquito larvae (Diptera: Culicidae) in Flanders (Belgium)

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Increasing globalization of transport of people and goods, together with climate change, create suitable conditions for the introduction of alien mosquito species as well as the (re)emergence of diseases transmitted by mosquitoes in Europe (1). Invading and alien species, as well as native species, can act as vectors of arboviruses (viruses transmitted by arthropods). Recent outbreaks of mosquito-borne diseases in Western Europe show the need for better knowledge of the taxonomic and functional biodiversity of both native and invasive vector species. To assess the distribution of adult mosquito species in Belgium a large scale inventory project was launched in 2007: the MODIRISK-project (1). This project resulted in an overview of the distribution and habitat niche of adult mosquitoes in Belgium.

However, larvae were neither collected nor surveyed and there is still a gap in information on larval-habitat characteristics of Belgian mosquitoes. Especially the niche of the larvae of some common species that are known as vector for arboviruses is still poorly known in Belgium and Flanders. Here, distribution maps for the encountered species are plotted and habitat requirements are investigated by assessing the circumstances in which the different taxa are found.

In the context of water quality monitoring by the Flemish Environment Agency, macroinvertebrates have been sampled at several thousand sampling points since 1989. However, it was only in 1997 that the sampling network became really extensive and therefore, material from 1997-2009 was studied. Since water quality monitoring has mainly been focusing on running waters, stagnant waters are underrepresented. During monitoring, macroinvertebrates were

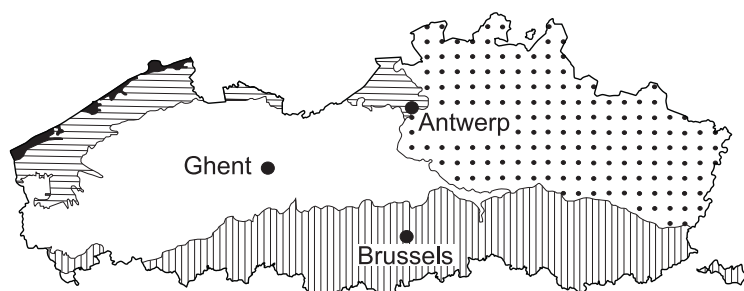


Fig. 1. – Map of Flanders with indication of the different ecoregions: dune area (black), polder area (horizontal stripes), sandy region (white), Campine region (dots) and loamy region (vertical stripes); the location of Flanders has been marked on the map.

sampled using a standard handnet, as described by GABRIELS et al. (2). At each sampling point, conductivity, pH, oxygen content and water temperature were measured. All sampled mosquito larvae were identified to the lowest possible level, which was in most cases possible up to the species, by using the identification key by SCHAFFNER et al. (3) and we used the traditional taxonomical classification (pre-REINERT, 4). In Flanders, a typology of the watercourses has been made by JOCHEMS et al. (5). The main separation of the types was based on the catchment area and in addition, the watercourses in the polder area were separated from the remaining watercourses and differentiation was made between small and large brooks from the different ecoregions: sandy region, the loamy region and Campine region. A map of Flanders with indication of the ecoregions is presented in Figure 1.

In the present study, 5823 mosquito larvae were identified, belonging to nine different taxa, however, only four species were encountered on more than three occasions. Distribution maps are given in Figure 2, while the water type where the different taxa were found from 1997-2009 is indicated in Table 1. *Anopheles*

*claviger* (MEIGEN, 1804) mostly occurred in small streams, but avoided polder watercourses. *Anopheles maculipennis s.l.* was also sampled in larger watercourses as well as polder watercourses and stagnant waters, but was rarely found in the loamy region. *Culex pipiens* LINNAEUS 1758 is a ubiquitous that was found in most water types, whereas *Culiseta annulata* (SCHRANK, 1776) occurred predominantly in small streams. *Aedes caspius* (PALLAS, 1771)/*dorsalis* (MEIGEN, 1830) was only found once in a brackish polder watercourse that was oversaturated with oxygen. *Culex territans* WALKER 1856, *Aedes punctor* (KIRBY, 1837) and *Aedes rusticus* (ROSSI, 1779) were only caught on a few occasions in small brooks, while *Culiseta morsitans* (THEOBALD, 1901) was only captured once in a large brook.

*Anopheles claviger* (median 5.6mg O<sub>2</sub>.L<sup>-1</sup>) and *An. maculipennis s.l.* (6.4mg O<sub>2</sub>.L<sup>-1</sup>) tolerated a wide range of oxygen concentrations, but *Cx. pipiens* (4.8mg O<sub>2</sub>.L<sup>-1</sup>) and *Cs. annulata* (2.9mg O<sub>2</sub>.L<sup>-1</sup>) even occurred at significantly lower oxygen concentrations (Kruskal-Wallis ANOVA followed by multiple comparisons). These four species all preferred slightly alkaline waters with a pH between 7 and 8 and tolerated a wide range

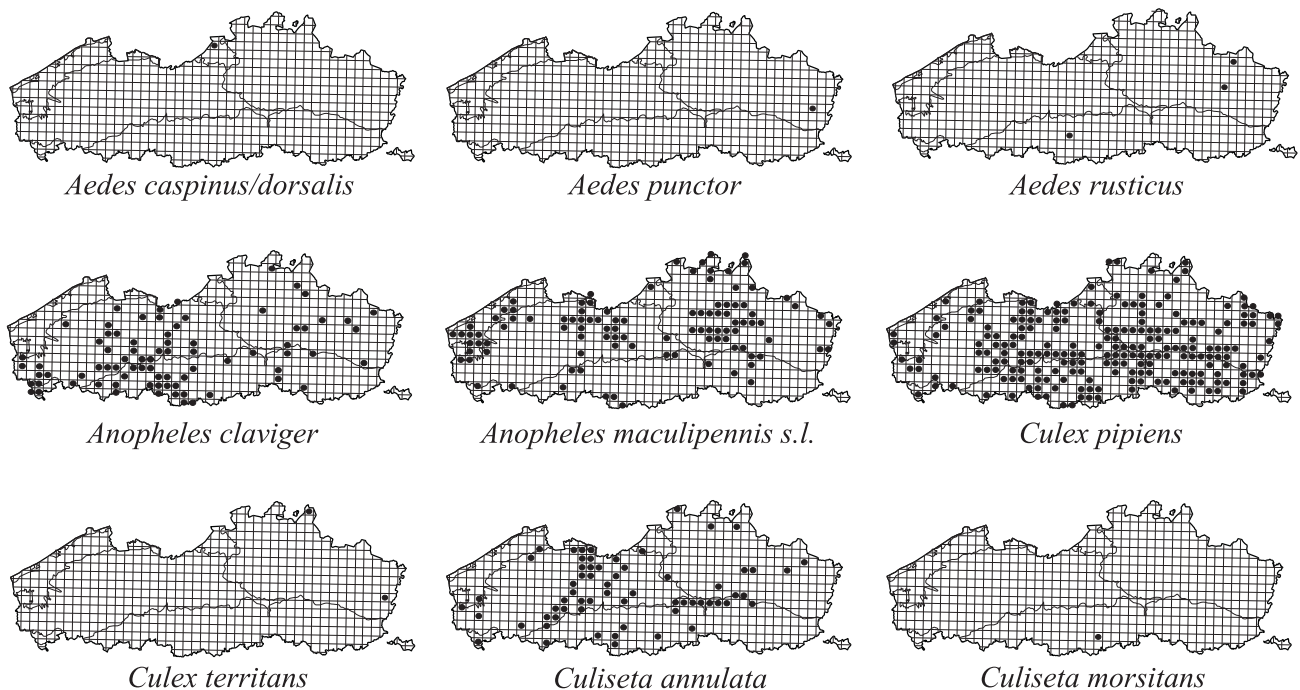


Fig. 2. – Distribution of the sampled mosquitoes in Flanders, with indication of the ecoregions and a grid of 5\*5 km UTM-squares.

Table 1

Number of records of mosquito larvae per water type in Flanders from 1997 to 2009.

Water type Ecoregion	Large river 600-10000	Small river 300-600	Large brook			Small brook			Polder water-course Polder	Circum-neutral lake 6.5-7.5	Alkaline lake >7.5
			Campine 50-300	Loamy 50-300	Sandy 50-300	Campine < 50	Loamy < 50	Sandy < 50			
<i>Aedes caspius/dorsalis</i>											
<i>Aedes punctor</i>						1			1		
<i>Aedes rusticus</i>	1	1				2	1				
<i>Anopheles claviger</i>			3	3	2	14	32	41		3	
<i>Anopheles maculipennis s.l.</i>	6	14	47	3	3	37	4	44	29	16	
<i>Culex pipiens</i>	5	6	18	20	12	66	90	121	15	4	
<i>Culex territans</i>						1				1	
<i>Culiseta annulata</i>		1			2	10	11	43	5	2	
<i>Culiseta morsitans</i>				1							

of conductivities, often surpassing  $1000\mu\text{S}\cdot\text{cm}^{-1}$ . However, some rarer species such as *Cx. territans*, *Ae. punctor* and *Ae. rusticus* were restricted to waters with a lower conductivity.

Only nine taxa were encountered during the present study, whereas 32 species have been reported from Belgium (6). However, only Flanders was studied and it is known that several species are restricted to Wallonia, the southern part of the country. In addition, some habitat types typical for mosquito larvae, such as temporal waters, tree holes, fens and peat boxes were not sampled, while stagnant waters were also underrepresented. Moreover, larvae of some species are often found in man-made habitats. During the present study, only surface waters (mainly running waters) were studied and data presented on the distribution and ecology of larvae will therefore inevitably be incomplete.

Some of the species encountered here are very widespread in all kinds of sampled waters and are potential vectors of arboviruses. Most interesting are *Cx. pipiens* and the two species of *Anopheles* that were found. The first, a potential vector for West Nile virus and very abundant and widespread, seems to have a wide range of potential larval breeding sites, including man-made breeding sites (7). In many regions in Flanders, West Nile virus is therefore a candidate for circulation in nature reserves but also near human settlements as it can be maintained in an avian-mosquito cycle (7). West Nile virus outbreaks are increasing in Central Europe (7) and due to global warming, these outbreaks can also be expected in Flanders in the near future. Studies on ecological delineation of the larval-breeding sites of *Anopheles* species are rare, although two taxa turned out to be widespread during the present study. *Anopheles claviger*, a primary vector of human malaria in the Middle-East, occurs in the Netherlands mostly in permanent pools, shallow canals and ditches, with a preference for shaded sites (7). Although Flanders is still too cold for human malaria, this could change if the temperature keeps rising due to global warming. Here, the larvae of *An.*

*claviger* were found in a wide range of water types, making this species also a top potential candidate as vector for Tahyna and Batai viruses in Flanders in the future (8). The *An. maculipennis* s.l. complex contains several species that are primary vectors for human malaria and different arboviruses. In the Netherlands, three species occur: *An. messeae*, *An. atroparvus* and *An. maculipennis* s.s. There, the species complex seems to occupy a wide range of water types and is widely distributed (9), which is also the case in Flanders, where it might act as a potential vector for different diseases.

Especially in some large nature reserves with high abundances of migratory birds during the mosquito season, some arboviruses can be maintained in an avian-mosquito cycle (7). In addition, there is a worldwide trend to construct, restore and protect wetlands and to improve the ecological water quality, which creates suitable habitats in which host, vectors and pathogens can come into contact. Thus at these sites, vectors might be exposed to pathogens (10). These areas, including wetlands, marshes and tidal zones, are often situated near urbanized areas (such as harbours), bringing introduced pathogens and vectors in close contact with humans. The knowledge on the larval ecology is important to better assess the areas where certain species can occur and how interventions such as creation of new wetlands, marshes and tidal zones will change mosquito species composition and abundance. This information can be used to design management strategies aiming at reducing the mosquito population with the objective to also reduce the transmission potential in a certain area. Here, we attempted to fill some gaps on knowledge about ecology of mosquito larvae in Flanders. This knowledge is indispensable if adequate control of mosquitoes becomes urgent during outbreaks of arboviruses or of malaria.

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