Short Communication

Human-Induced Expanded Distribution of *Anopheles plumbeus*, Experimental Vector of West Nile Virus and a Potential Vector of Human Malaria in Belgium

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ABSTRACT For the majority of native species, human-created habitats provide a hostile environment that prevents their colonization. However, if the conditions encountered in this novel environment are part of the fundamental niche of a particular species, these low competitive environments may allow strong population expansion of even rare and stenotopic species. If these species are potentially harmful to humans, such anthropogenic habitat alterations may impose strong risks for human health. Here, we report on a recent and severe outbreak of the viciously biting and day-active mosquito *Anopheles plumbeus* Stephens, 1828, that is caused by a habitat shift toward human-created habitats. Although historic data indicate that the species was previously reported to be rare in Belgium and confined to natural forest habitats, more recent data indicate a strong population expansion all over Belgium and severe nuisance at a local scale. We show that these outbreaks can be explained by a recent larval habitat shift of this species from tree-holes in forests to large manure collecting pits of abandoned and uncleaned pig stables. Further surveys of the colonization and detection of other potential larval breeding places of this mosquito in this artificial environment are of particular importance for human health because the species is known as a experimental vector of West Nile virus and a potential vector of human malaria.

KEY WORDS Culicidae, *Anopheles plumbeus*, habitat shift, larval collection, outbreaks

Novel artificial habitats differ in many respects from the conditions encountered in the natural environment. Due to their recent nature, ecological communities do not constitute historically authenticated coevolved species assemblages as is the case elsewhere in undisturbed habitats. Hence, the majority of native species in the surrounding habitats are not able to invade the unusual conditions encountered in these habitats (Shochat et al. 2006, Seastedt et al. 2008). However, if native species are able to cope with the abiotic and biotic conditions present in these novel artificial habitats, the low competitive environment of these habitats might lead to favorable conditions for strong population expansion of previously less common species (Hobbs et al. 2009). In parallel with the recent expansion of these artificial habitats by human activities, interest by ecologist in these ecosystems is beginning to emerge. This is of particular relevance if species that are part of these ecosystems impose serious problems on, e.g., the ecosystem level, when mass effects deteriorate ecosystem functioning in neighboring habitats or when they are a potential risk for human health (Simon et al. 2008).

Anopheles plumbeus Stephens, 1828, is a mosquito that is historically reported from forests where it breeds in permanent wet cavities of trees and water-filled holes of old trees (Bradshaw and Holzapfel 1991, Snow 1998). It occupies permanent rot holes and also may extend habitat use into deeper, more cryptic buttress holes in beech trees (Bradshaw and Holzapfel 1992). Occasionally, it develops in containers with stagnant rain water and groundwater (Marshall 1938). Eggs are laid just above the waterline level, and they hatch when first flooded (Service 1968). According to historic descriptions on its distribution and habitat preference, *An. plumbeus* was not recorded in Belgium before 1925 (Goetghebuer 1925). A reevaluation of the Royal Belgian Institute of Natural Sciences mosquito collections (unpublished data) revealed that the

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Fig. 1. Former records and present distribution of *An. plumbeus* in Belgium. Black dots, records 2007–2008; gray dots, records <1975.

first record of *An. plumbeus* goes back to 12 July 1938 when Goetghebuer collected this species in Rouvroy near Torgny. Between its first discovery in Torgny and 1975, this species was only occasionally collected in Belgium, and the majority of these records stem from highly forested regions (Fig. 1).

From 2008 onward, cases of extreme nuisance caused by massive abundances of day-active *An. plumbeus* were reported at >10 suburban sites (gardens and near farms) in Flanders, Belgium. In parallel, distributional data obtained within the framework of the MODIRISK-project (see www.modirisk.be) suggested that the species shifted its habitat from forests to anthropogenic influenced habitats, which greatly enlarged its geographic distribution (Fig. 1). Similar patterns are observed in other western European countries where larvae were able to live in artificial breeding sites, such as tires (Karch 1996, Schaffner et al. 2001, Takken et al. 2007).

The ecological conditions resulting in the recent and rapid population expansion of this species in urban areas are however still unknown. Besides nuisance at local scale, a strong impact on human health at a larger scale is to be expected because *An. plumbeus* is a reported laboratory vector of West Nile (Vermeil et al. 1960, Schaffner et al. 2001). Moreover, experiments showed that *An. plumbeus* is susceptible to *Plasmodium falciparum* malaria at least to the oocyst stage (Blacklock and Carter 1920, Marchant et al. 1998) and that it was probably involved in the occurrence of two cases of autochthonous *P. falciparum* malaria in Germany (Kruger et al. 2001).

Materials and Methods

To demonstrate the expanded present distribution rate of *An. plumbeus,* distribution data of mosquitoes obtained during the MODIRISK-project were listed. During this large-scale mosquito inventory, the Corine Land Cover (2000) classification (JRC-IES 2005) was used to delineate potential mosquito habitats. This data layer was overlaid with the Military Grid Reference System (MGRS) that is used internationally for species mapping, such as for mammals (Amori et al. 2002) and birds (Hagemeijer and Blair 1997). Across Belgium, 312 10- by 10-km MGRS cells were identified. An average of three points was sampled per cell, whereas the number of points per aggregated cell was proportional to its total surface. In total, 936 sites were selected from three key habitats in Belgium (urban, $n = 173$; agriculture, $n = 564$; and natural, $n = 199$). From 8 May to 5 October 2007, 488 sites were sampled with a Mosquito Magnet Liberty Plus (further called MMLP) for 1 wk. In summer 2008, 488 other sites were sampled with MMLP for 1 wk.

Table 1. Surveyed PBSs at the *An. plumbeus* pest site and the average number of larvae collected during one survey

Code of the site	Description of site	*n* larvae/survey[a]		
		ANO PLU	CLX PIP	CUL ANN
PBS01	Pond in sheep meadow		17	
PBS02	Small brooklet along small path in fields		8	
PBS03	Small brooklet at the side of the road		12	6
PBS04	Small brooklet at the side of the road		1	10
PBS05	Very small brooklet near agricultural (maize) field		1	4
PBS06	Very small brooklet near agricultural (maize) field			
PBS07	Very small brooklet near agricultural (maize) field			1
PBS08	Very small brooklet near garden			
PBS09	Very small brooklet near garden and compost heap			
PBS10	Lower and uppercase cavity in Willow trees along the road			
PBS11	Small second used tyre		6	
PBS12	Large second used tyre		7	
PBS13	Very large second hand tyre		3	19
PBS14	Pond in garden with fish			
PBS15	Pond in garden with fish	2		
PBS16	Subterranean manure collection tank in pig stable	105	4	
PBS17	Manure pit of pig stable	52	19	
PBS18	Sewerage along the road	1	3	
PBS19	Artificial breeding container 1 in shady, closed vegetation		28	
PBS20	Artificial breeding container 2 in sunny, open vegetation		9	
PBS21	Artificial breeding container 3 in shady, closed vegetation		18	
PBS22	Artificial breeding container 4 in sunny, open vegetation		17	5
PBS23	Artificial breeding container 5 under hedge		18	
PBS24	Artificial breeding container 6 in shady, closed vegetation		12	
PBS25	Artificial breeding container 7 in sunny, open vegetation		24	
PBS26	Artificial breeding container 8 in sunny, open vegetation		13	2

[a] ANO PLU, *An. plumbeus;* CLX PIP, *Cx. pipiens;* and CUL ANN, *Cs. annulata.*

To unravel potential larval breeding places of *An. plumbeus* in urban habitats and to monitor adult mosquitoes during one summer at a pest site, we surveyed from May 2009 onward one of the sites where outbreaks of *An. plumbeus* were reported. We therefore screened all potential natural and human-created habitats, hereafter called potential breeding sites (PBSs), that could act as breeding sites at one particular locality in Torhout (51° 04′13.84″ N, 3° 07′33.59″ E), situated 350 m of the Groenhovebos forest complex (Table 1). In each PBS, a net (12 cm in width, 17 cm in length) and 500-ml capacity dipper were used three times per survey. We explored nine potential natural mosquito breeding sites (PBS01–10) at two different periods (beginning of July and end of September) in 2009 and eight human-created potential breeding sites in September 2009 (PBS11–18). Moreover, eight artificial breeding containers (small flower pots with ø = 18 cm, height = 20 cm) were installed at the study site (four PBS at each edge of the garden in one line, 20 m separated form each other) in June 2009 (PBS19–26) and inspected simultaneously with the other PBS. They were filled with an infusion-baited mixture for gravid traps, made by placing ≈0.075 kg of fresh grass clippings or hay into a 10-liter plastic trash can and supplied with 0.5 g of brewers yeast and 10 liters of tap water. Before filling the artificial breeding containers, both cans were left in a sunny location to allow a 5–7-d fermentation of the mixture (Scott et al. 2001).

To monitor the phenology of adult mosquitoes during spring and summer, two MMLP (MMLP01 and MMLP02) were installed at the pest site, which operated 48 h, fortnightly starting at 1200 hours on 20 April 2009 and ending 10 October 2009. The traps were emptied 13 times.

Results

During the MODIRISK-project, *An. plumbeus* was found at 114 localities spread over Belgium (Fig. 1). We retrieved 30 records from urban habitats (17.3% of all sampled urban), 47 in agriculture habitats (8.3% of all sampled agricultural sites), and 37 in natural habitats (18.6% of all sampled natural sites).

Larvae of three different mosquito species were found at the different PBS sampled in the current study (Table 1). Besides *Culex pipiens* L., found in 73% of the surveyed breeding sites, *Culiseta annulata* Schrank larvae (seven PBS) were present in both natural and human-created PBSs. *An. plumbeus* larvae were found in only four surveyed sites, albeit in often large numbers. The most important breeding site was found in an abandoned yet uncleaned pig stable, where larvae develop in subterranean manure collection tanks in the water on top of the manure. Here, >30 larvae were found in a single net sweep. Nevertheless, smaller larval numbers were found in PBS15 and PBS18, indicating that females also used other human created PBS to lay eggs. Although present at the study site in Torhout, second-hand tires (PBS11–13) were not colonized by *An. plumbeus.* Nevertheless, studies conducted by Schaffner et al. 2004 and Versteirt et al. 2009 demonstrated that they are a potential human-induced breeding site for this mosquito species elsewhere in Belgium.

Table 2. Number of adult *An. plumbeus* collected fortnightly, during 48 h from 20 April 2009 until 7 October with Mosquito Magnet Liberty Plus trap

Start date	End date	MMPL01	MMPL02
20 April 2009	22 April 2009	0	0
4 May 2009	6 May 2009	1	0
18 May 2009	20 May 2009	754	1,224
1 June 2009	3 June 2009	182	5,034
15 June 2009	17 June 2009	728	7,121
29 June 2009	1 July 2009	1,471	2,054
13 July 2009	15 July 2009	2,038	442
27 July 2009	29 July 2009	513	924
10 Aug. 2009	12 Aug. 2009	1,785	1,132
24 Aug. 2009	26 Aug. 2009	180	340
7 Sept. 2009	9 Sept. 2009	631	663
21 Sept. 2009	23 Sept. 2009	101	0
5 Oct. 2009	7 Oct. 2009	0	0

Traps (MMLP01 and MMLP02) were installed at each edge of the garden, 50 m separated from each other.

Near this novel human-induced breeding site and in neighboring gardens, adults were found quickly biting humans and they preferred resting in places shaded with trees and hedges (unpublished data). Adults were collected from the beginning of May until the beginning of September for MMLP02 and from the end of September for MMLP01 (Table 2). Large numbers of adult *An. plumbeus* were collected in the beginning of June for MMLP02 (5,034 during 2 d in beginning of June and 7,121 by mid-June) and mid-July and mid-August for MMLP01 (2,038 and 1,785, respectively).

Discussion

Our observations indicate that abandoned manure tanks might serve as favored breeding sites of *An. plumbeus* that allow development of large numbers of larvae during summer. These abandoned and uncleaned stables are neglected by the owners. Hence, roofs break down and rain water is gathered in the manure pits. Moreover, all stables have a surface area of 10 by 30 m and have (in most cases) one big collecting pit over the complete area of the stable. These pits are 1 m deep and a large water volume is available for the mosquito larvae. These manure pits once colonized, allow *An. plumbeus* to reach very high larval densities in these huge water volumes (10 by 30 by 1 m volume) compared with small tree-holes and used tires (only a few liters water).

Near these pig stables (gardens of neighbors) and within this human made ecosystem, adult *An. plumbeus* reach large densities that may pose serious nuisance to humans from May to September. This might pose particular problems in several regions in Flanders where many owners of pig stables recently received European Union-funds to run down their pig-rearing activities. Hence, many so far not-yet-colonized manure pits await colonization by *An. plumbeus.* This habitat shift of *An. plumbeus* could result in pest outbreaks in certain regions in Belgium and eventually could be responsible for autochthonous malaria cases. Moreover, the large population densities might facilitate colonization success of other novel human-created habitats near the abandoned pig stables. Potential sites with similar conditions are manure pits of compost mounds, sewerage with stagnant water, and abandoned manure pits of stables of small cattle. Although they might not be as important and large as the manure tanks of abandoned pig stables, they might be sufficient to alter the colonization–extinction balance (Levins 1969, Hanski 1991) and could lead to a persistent presence of the species in anthropogenic influenced habitats even after removal of the core breeding site.

Besides its relevance for human health, *An. plumbeus* offers an ideal model organism to investigate the mechanisms that underlie shifts toward novel habitats. It remains unknown whether the colonization toward this novel habitat implies ecotypic differentiation, as has been observed for populations of the *Cx. pipiens* in response to breeding in subterranean and human-made sites (hypogeous habitat) from natural populations across Europe (Chevillon et al. 1998, Byrne and Nichols 1999). Also, studies from the Camargue region suggests that anthropogenic changes should not be underestimated in vectorborne disease recrudescence (Ponçon et al. 2007). Understanding processes caused by habitat shifts as is the case with *An. plumbeus,* is of increasing importance when it induces the spread of species that have economic implications or a strong impact on human health.

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