Full Length Research Paper

Distribution and characterization of anopheline larval habitats in flooded areas of the Dakar suburbs (Senegal)

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Knowledge of mosquito ecology is a prerequisite for efficient implementation of vector control strategies. This one-year study was carried out during the 2013 rainy season and the 2014 dry season throughout the flooded areas of the suburbs of Dakar with the aim to characterize and map anopheline larval habitats. In both seasons, all water bodies that were encountered within the study departments were geo-located and their features (type of water body, size, turbidity and distance to human dwellings) recorded. The presence of anopheline and/or culicine larvae and predators was assessed. A total of 908 and 278 potential larval habitats were visited during the rainy season and the dry season, respectively. A significant positive association was found between the rainy season and the presence of anopheline larval sites, which consisted of ponds, puddles, ravines, drain channels, streams and canals. Anopheline larvae were more likely to be found in clear water bodies located within 10 m to human dwellings. During the dry season, only puddles were likely to host anopheline larvae. Anopheline larval habitats were significantly more frequent in the Department of Rufisque during the rainy season (univariate analysis, $P = 0.006$) and in the Department of Guediawaye during the dry season (multivariate analysis, $P = 0.036$). The malaria vector identified was *Anopheles arabiensis*. Data gathered in this study will guide larval control programmes in urban settings prone to flooding.

Key words: Anopheline, larval habitat, flood, suburb of Dakar, Senegal.

INTRODUCTION

Despite a decade of control efforts and a 54% decrease in malaria deaths (between 2000 and 2013), malaria remains a serious public health problem across sub-Saharan Africa (World Health Organization (WHO), 2014). In 2013, 198 million malaria cases leading to 584,000 deaths were recorded in the continent. Malaria

transmission is heterogeneous in nature (Bousema et al., 2012; Opondo et al., 2016). Among the multiple factors creating this heterogeneity is urbanization which leads to new control challenges (Dongus et al., 2009; Donnelly et al., 2005; Klinkenberg et al., 2008). Many African cities face rapid and uncontrolled urbanization that is worsening the socio-economic and environmental conditions (RGPHAE, 2013; World Urbanization Prospects (WUP), 2014). Although urban areas are normally characterized by low malaria burden (Robert et al., 2003), unplanned urbanization can lead to the creation of new larval habitats that can potentially produce more vectors and hence contribute to malaria transmission (Dongus et al., 2009).

The suburbs of Dakar, the capital city of Senegal is an area where malaria transmission persists all year long within some localities (Diallo et al., 2012; Programme National de Lutte contre le Paludisme (PNLP), 2013) despite the mass distribution of insecticide-treated nets (ITNs), as well as, introduction of artemisinin-based combination therapy (ACT). The non-structured urbanization and recurrent flooding observed in these areas facilitate the proliferation of natural and/or man-made anopheline larval habitats. Dakar's suburbs are facing huge environmental issues due to the presence of such spontaneous and irregular habitats in these floodplains and they have led to critical sanitation problems throughout the year. This situation has allowed the proliferation of malaria and arbovirus vectors. The development of anopheline immature stages usually occurs in fresh or brackish non-polluted water, with or without vegetation, exposed or not to the sun (Carnevale and Robert, 2009). However, recent studies showed that *Anophelese gambiae* sensu lato can adapt to extreme conditions and may grow in a wide variety of man-made larval sites, sometimes polluted by industrial, agricultural or domestic runoffs. Some anopheline mosquitoes have thus diversified their larval sites, from previously clear, unpolluted waters to include more polluted stagnant water sources (Djouaka et al., 2007; Gunathilaka et al., 2013, 2014; Gunathilaka and Karunaraj, 2015; Keating et al., 2004).

The use of ITNs and indoor residual spraying (IRS) are the principal vector control strategies deployed to stop the spread of malaria transmission (Bhatt et al., 2015). In addition to ITNs and IRS, mosquito larval source management (LSM) has been recommended as a complementary tool for vector control strategies (WHO, 2013) where it has been successful in the control and elimination of certain vectors in Brazil (Soper and Wilson, 1943), in the United States of America (Carlson, 2006; Gadawski, 1989) and throughout Europe (Becker, 2010).

Fillinger and Lindsay (2011) provided evidence of the efficiency of the LSM for malaria control in Africa based on trials in areas where all larval habitats were well defined and accessible. These results give evidence that LSM can be used to complement and strengthen core vector control interventions to further reduce malaria transmission in an urban area such as Dakar and its suburbs (Fillinger and Lindsay, 2011; RBM, 2011; WHO, 2013). However, an accurate knowledge of malaria vector larval ecology is a prerequisite before implementing any effective LSM strategy.

Few studies have investigated the dynamics of anopheline larval sites throughout the suburbs of Dakar (Awono-Ambene and Robert, 1999; Gadiaga et al., 2011; Robert et al., 1998) and none, to our knowledge, has assessed the consequence of the recurrent flooding on the larval habitats of malaria vector species. Therefore, the aim of this study was to map and to characterize anopheline larval habitats in the suburbs of Dakar.

MATERIALS AND METHODS

Study area

The study was carried out in the Dakar region of Senegal located at the westernmost part of the Cape-Verde peninsula (14°40'20"N, 17°25'22"W). Dakar belongs to the Sudano-Sahelian domain and is characterized by specific eco-geographical areas called "Niayes", where the presence of sand dune depressions and a flushing water table make them prone to flooding during the rainy season. The region essentially has two seasons: a hot, rainy season from July to October (with average temperatures ranging from 24 to 30°C) and a mild cold, dry season from November to June (with average temperatures ranging from 19 to 25°C). The annual rainfall was 566 mm in 2013 (ANAMS, 2013). The Dakar region is the most populated area of Senegal with a density of 5,404 inhabitants per km² (RGPHAE, 2013).

Selection of study sites

The region is divided into four departments; Dakar, Guediawaye, Pikine and Rufisque and this study was undertaken in all except Dakar. Flooding is common in particular localities within the departments during the rainy season. Thus, in each of them, both flooded and non-flooded areas have been investigated. These three departments with different population densities have experienced dramatic changes in demography as well as unplanned and uncontrolled urban expansion. The department of Guediawaye is 17.8 km² with a population density of 18,541 inhabitants per km². The department of Pikine with 14,472 inhabitants per km² has a surface area of 80.9 km². Rufisque is the largest department (379.6 km²) with the lowest population density, at 1,292 inhabitants per km². However, within Rufisque department, only the urban area was investigated as its population density (10,511 inhabitants per km²) is comparable to those of Pikine and Guediawaye

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Table 1. Sampling of anopheline larval habitats selected for a characterization of the species.

| Type of larval habitats | Rainy season | | | | | | Dry season | | | | | |
| | Pikine | | Guediawaye | | Rufisque | | Pikine | | Guediawaye | | Rufisque | |
	N[a]	n[b] (%)	N	n (%)	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Basin	3	1	1	1	0	0	0	0	0	0	0	0
Canals	5	5	0	0	13	5	0	0	0	0	2	2
Market-garden wells	4	4	2	1	0	0	0	0	0	0	0	0
Puddles	21	19	14	4	32	3	2	2	2	2	3	3
Lakes	4	4	0	0	0	0	0	0	0	0	0	0
Flooded houses	137	49	27	2	12	1	10	10	2	2	0	0
Ponds	218	37	15	0	15	0	14	9	2	2	1	1
Backwaters	1	0	0	0	0	0	0	0	0	0	0	0
Wells	2	1	0	0	1	1	0	0	0	0	1	1
Ravines	3	3	1	0	9	0	0	0	0	0	0	0
Drain channels	1	1	0	0	18	2	0	0	0	0	0	0
Streams	0	0	2	1	12	1	0	0	0	0	0	0
Holes	0	0	0	0	2	0	0	0	0	0	0	0
Total	399	124 (31)	62	9 (15)	114	13 (11)	26	21 (81)	6	6 (100)	7	7 (100)

[a]All larval habitats visited. [b]Number of larval habitats selected for the identification of the species present.

(ANAT, 2016; RGPHAE, 2013).

Investigation and characterization of water bodies

The study was conducted during the 2013 rainy season from July to October and the 2014 dry season from March to June. Within each department, all water bodies encountered were geo-located using a Garmin eTrex® GPS (Legend H), listed then characterized according to type, size, depth, turbidity and exposure to sunlight. The distance between each encountered water body and the nearest human dwelling and the presence/absence of predators (tadpoles and fish) were also recorded. Each water body was inspected once per season and was mapped using ArcGIS version 9.1 software.

Larval collection, sampling and identification

The surface and edges of water bodies were checked at different places for the presence of mosquito larvae. The immature stages of mosquito populations from positive water bodies of each study area were collected using the standard dipping method (Service, 1993). The anopheline larvae were separated from the culicines (Carnevale and Robert, 2009). The anopheline larvae were then transferred to the laboratory in buckets filled water from the site where the larvae were collected. Anopheles were identified to species level from a random selection of 146 and 34 anopheline larval habitats from the three departments during 2013 rainy season and 2014 dry season, respectively (Table 1). Larvae from the selected larval habitats were reared to obtain adults. These were pooled according to the type of larval habitat, the department of origin and the season, and allowed to emerge. A total of 2447 adult were obtained. Half (1226/2447), distributed according to the type of larval habitat, to the department and to the season were identified morphologically (Diagne et al., 1994). In each stratum composing 1226 anopheles morphologically identified, a random sample proportional to the size (Loonis, 2009) of the original population was made in order to have a total of 638 specimens for molecular identification of the species (Wilkins et al., 2006).

Data analysis

All statistical analyses were performed using STATA 11 software (Stata Corp-LP®). Logistic regression was used to investigate the effect of geographic (department) and bio-ecological factors (season, type, water turbidity, depth, proximity to human dwellings, size, sunlight and the presence of predatory species) on presence/absence of anopheline larvae. For each variable, the modalities poorly represented (<5) or those in which the absence of anopheline larvae was noted, were not included in the regression analyses. Variables with a p-value strictly lower than 0.2 for the simple regression analyses were integrated in multiple regression analyses. A stepwise logistic regression analysis was done and variables removed sequentially based on Akaike information criterion (AIC) till the best model was achieved. The significance level was fixed at α = 0.05 in the final model. Proportion of anopheline and/or culicine larvae per season was assessed using Chi-square tests.

RESULTS

Water body survey

During the whole survey, a total of 908 water bodies, consisting of 13 types of water bodies, which varied in size, depth and exposure to the sun, were identified (Supp.S1). Of the overall 908 that were present during the rainy season, only 278 were encountered during the dry season. Eighty three percent (759/908) of water bodies were positive for mosquito larvae during the rainy season versus 55% (154/278) throughout the dry season. Positive water bodies were composed of anopheline, culicine or mixed (anopheline and culicine) larval habitats. In the rainy season, anopheline larvae (anopheline exclusively and mixed) colonized a higher proportion of water bodies (63%) than the culicine larvae

Table 2. Number of water bodies surveyed.

Survey period	Water bodies	Larval habitats					
		anopheline	%	culicine	%	[a]mixed	%
Rainy season 2013	908	271***	30	184***	20	304***	33
Dry season 2014	278	20***	7	115***	41	19***	7

[a]Anopheline and Culicine larvae. Chi-square test: Within a column, an asterisk indicates significant differences (P < 0.05) between the lines (*, P < 0.05; **, P < 0.01; ***, P < 0.001; NS, not significant).

Table 3. Factors associated with the presence of anopheline larvae during the 2013 rainy season

Characteristics	Variables	Number of water bodies	[a]Anopheline larval habitats n (%)	Univariate analysis		Multivariate analysis	
				[b]OR ([c]95% CI)	P-value	[d]AOR (95% CI)	P-value
Department	Pikine	662	399 (60)	1			
	Guediawaye	88	62 (70)	1.57 (0.97 2.55)	0.067		
	Rufisque	158	114 ((72)	1.71 (1.17 - 2.50)	0.006*		
Types of water bodies	Basins	12	4 (33)	1		1	
	Canals	30	18 (60)	3.37 (0.84 - 13.49)	0.085	5 (1.15- 21.85)	0.032*
	Market-garden wells	8	6 (75)	6.75 (0.92 - 0.23)	0.060	5.5(0.73 - 42.69)	0.097
	Puddles	89	67 (75)	6.85 (1.92 -24.46)	0.003*	6.6 (1.72 - 25.38)	0.006 *
	Lakes	11	4 (36)	1.28 (0.23 - 7.05)	0.772	1.14 (0.19 - 6.79)	0.879
	Flooded houses	286	176 (62)	3.6 (1.08 - 11.97)	0.037*	3.04 (0.85- 10.91)	0.088
	Ponds	404	248 (61)	3.58 (1.08 - 11.81)	0.037*	3.68 (1.04 - 13.11)	0.044*
	Backwaters	1	1 (100)	-		-	
	Wells	8	3 (38)	1.35 (0.21 - 8.62)	0.751	0.99 (0.14 - 6.77)	0.991
	Ravines	16	13 (81)	9.75 (1.74 - 54.52)	0.010*	7.18 (1.2 - 43.05)	0.031*
	Drain channels	22	19 (86)	14.25 (2.62 - 77.54)	0.002*	13.87 (2.32 - 82.88)	0.004*
	Streams	19	14 (74)	6.3(1.32 - 29.94)	0.021*	5.55 (1.08 - 28.56)	0.040*
	Holes	2	2 (100)	-		-	
Water turbidity	Clear water	415	313 (75)	1		1	
	+/- Turbid water	340	209 (61)	0.52 (0.38 - 0.71)	< 0.001*	0.52 (0.37 - 0.72)	< 0.001*
	Turbid water	153	53 (35)	0.17 (0.11 - 0.25)	< 0.001*	0.16 (0.11 - 0.24)	< 0.001*
Depth (centimeters)	≤ 50	555	372 (67)	1			
	> 50	353	203 (58)	0.66 (0.50 - 0.88)	0.004		

(culicine exclusively and mixed) (53%) (χ^2= 17.17; P < 0.001). An opposite trend was observed during the dry season where culicine larval sites (culicine exclusively and mixed) were more frequent (48%) (χ^2 = 75.73; P < 0.001) compared to those sites harbouring anopheline larvae (anopheline exclusively and mixed) (14%) (Table 2). The anopheline larval sites were 13 times more frequent during the rainy season (odds ratio [OR] = 12.99; P < 0.001) than the dry season (Table 3). The presence of anophelines larvae did not have an effect on the presence of culicines larvae in both the rainy (OR = 0.91; P = 0.487) and dry season (OR = 1.02; P = 0.944) (Tables 3 and 4).

Characterization of anopheline larval habitats

During the survey, thirteen types of water bodies were identified corresponding to the basins, market-garden wells, canals, puddles, lakes, flooded houses, ponds, backwaters, wells, ravines, drains channels, streams and holes. Overall, ponds, flooded houses, puddles and canals were the most frequent water bodies encountered during the survey. During the rainy season, all holes (2/2) and backwaters (1/1) were colonized by anopheline larvae and 86% (19/22) of drain channels, 81% (13/16) of ravines, 75% (67/89) of puddles, 75% (6/8) of market-garden wells, 74% (14/19) of streams, 62% (176/286) of

Table 3. Factors associated with the presence of anopheline larvae during the 2013 rainy season (continued).

Characteristics	Variables	Number of water bodies	Anopheline larval habitats n (%)	Univariate analysis		Multivariate analysis	
				OR ([c]95% CI)	P-value	AOR (95% CI)	P-value
Proximity (meters)	> 10	76	39 (51)	1		1	
	≤ 10	832	536 (64)	1.72 (1.07 - 2.75)	0.025	1.87 (1 - 3.17)	0.020
Size (m²)	0 - 100	227	166 (73)	1			
	100 - 500	468	288 (62)	0.59 (0.41 - 0.83)	0.003		
	500 - 1000	108	65 (60)	0.55 (0.34 - 0.90)	0.017		
	>1000	105	56 (53)	0.41 (0.25 - 0.68)	< 0.001		
Sunlight	No shade	18	10 (56)	1			
	Partial shade	10	8 (80)	3.20 (0.52 - 19.49)	0.207		
	Full shade	880	557 (63)	1.37 (0.54 - 3.53)	0.502		
culicine larvae	Absent	420	271 (64)	1			
	Present	488	304 (62)	0.91 (0.70 - 1.20)	0.487		
Tadpoles	Absent	477	320 (67)	1			
	Present	431	255 (59)	0.71 (0.54 - 0.93)	0.014		
Fish	Absent	848	541 (64)	1			
	Present	60	34 (57)	0.74 (0.43 - 1.26)	0.269		
Season	Dry season	278	39 (14)	1			
	Rainy season	908	575 (63)	12.99 (9.01 - 18.74)	< 0.001		

[a]anopheline larvae exclusively and mixed; [b]OR = Unadjusted Odds Ratio; [c]95% OR Confidence Interval; [d]AOR = Adjusted Odds Ratio.

flooded houses, 61% (248/404) of ponds and 60% (18/30) of canals were also positive for anopheline larvae (Table 3).

The univariate analysis showed that during the rainy season, flooded houses (OR = 3.6; P = 0.037), ponds (OR = 3.58; P = 0.037), puddles (OR = 6.85; P = 0.003), ravines (OR = 9.75; P = 0.010), drain channels (OR =14.25; P = 0.002) and streams (OR = 6.3; P = 0.021) were more likely to be colonized by anopheline larvae. Furthermore, in the multivariate analysis, ponds (adjusted OR [AOR] = 3.68; P = 0.044), puddles (AOR = 6.6; P = 0.006), ravines (AOR = 7.18; P = 0.031), drain channels (AOR = 13.87; P = 0.004), streams (AOR = 5.55; P = 0.040) and canals (AOR = 5; P = 0.032) were all positively associated with the presence of anopheline larvae (Table 3). During the dry season, anopheline larvae were scarce and only found in 47% (7/15) of puddles, 25% (1/4) of wells, 16% (17/106) of ponds and 12% (12/100) of flooded houses (Table 4). The presence of anopheline larvae was significantly associated with the puddles in univariate (OR = 9.18; P = 0.014) and multivariate (AOR = 19.48; P = 0.005) analyses during this season (Table 4).

The majority of water bodies (92%) were within 10 m of human dwellings. During the rainy season, 64% (536/832)

of these water bodies hosted anopheline larvae. The regression analysis showed that water bodies within 10 m of houses were positively associated with the presence of anopheline larvae both in the univariate analysis (OR = 1.72, P = 0.025) and the final multivariate model (AOR = 1.80, P = 0.029) (Table 3).

There was a negative association between water turbidity (Figure 1) and presence of anopheline larvae both in univariate (OR = 0.17, P < 0.001) and multivariate (AOR = 0.16, P < 0.001) analyses. Presence of predators (tadpoles) also had a negative correlation with presence of anopheline larvae (univariate analysis, OR = 0.71, P = 0.014) (Table 3).

Both size and depth of the bodies of water appeared to influence the presence of anopheline larvae. During the rainy season, medium (100 to 500 m²) and large (>500 m²) water bodies harboured anopheline larvae less frequently than water bodies of a smaller size (<100 m²) (OR = 0.59, P = 0.003) (Table 3). At the same time, the deepest water bodies were less favorable for the presence of anopheline larvae (OR = 0.66, P = 0.004) (Table 3). Conversely, in the dry season, no association was found between anopheline larvae presence and size or depth of water bodies (OR = 0.90, P = 0.778) (Table 4). During this dry season, proximity to human dwelling,

Table 4. Factors associated with the presence of anopheline larvae during the 2014 dry season.

Characteristics	Variables	Number of water bodies	Anopheline larval habitats n (%)	Univariate analysis		Multivariate analysis	
				OR (95% CI)	p-value	AOR (95% CI)	P-value
Department	Pikine	220	26 (12)	1			
	Guediawaye	23	6(26)	2.63 (0.95 - 7.28)	0.062	3.27 (1.08 - 9.89)	0.036
	Rufisque	35	7 (20)	1.86 (0.74 - 4.70)	0.186	3.19 (0.81 - 12.57)	0.097
[a]Types of water bodies	Basins	13	0 (0)	-			
	Market-garden wells	2	0 (0)	-			
	Backwaters	1	0 (0)	-			
	Wells	4	1 (25)	-			
	Ravines	2	0 (0)	-			
	Lakes	8	0 (0)	-			
	Drain channels	2	0 (0)	-			
	Streams	2	0 (0)	-			
	Canals	23	2 (9)	1			
	Puddles	15	7 (47)	9.18 (1.56 - 53.93)	0.014	19.48 (2.48 - 153.01)	0.005
	Flooded houses	100	12 (12)	1.43 (0.3 - 6.89)	0.654	2.87 (0.41 - 19.93)	0.286
	Ponds	106	17 (16)	2 (0.43 - 9.36)	0.376	4.39(0.67 - 28.83)	0.124
Water turbidity	+/- Turbid water	218	39 (18)	-			
	Turbid water	60	0 (0)	-			
Depth (centimeters)	≤ 50	187	27 (14)	1			
	> 50	91	12 (13)	0.90 (0.43 - 1.87)	0.778		
Proximity (meters)	>10	26	1 (4)	1		1	
	≤ 10	252	38 (15)	4.44 (0.58 - 33.74)	0.150	7.37 (0.76 - 71.5)	0.085
Size (m²)	0 - 100	68	12 (18)	1			
	100 - 500	140	20 (14)	0.78 (0.35 1.70)	0.529		
	500 – 1000	40	5 (13)	0.67 (0.22 - 2.05)	0.480		
	>1000	30	2 (7)	0.33 (0.07 - 1.59)	0.169		
Sunlight	No shade	13	1(8)	1			
	Partial shade	2	0 (0)	-			
	Full shaded	263	38 (14)	2.02 (0.26 - 16.04)	0.503		
Culicine larvae	Absent	144	20 (14)	1			
	Present	134	19 (14)	1.02 (0.52 - 2.02)	0.944		
Tadpoles	Absent	133	21 (16)	1			
	Present	145	18 (12)	0.75 (0.38 - 1.49)	0.419		
Fish	Absent	227	33 (15)	1			
	Present	51	6 (12)	0.78 (0.31 - 1.98)	0.607		

[a]In the dry season for the variable "type of water bodies", the reference is canals instead of basin because of the absence of anopheline larvae in the basin.

turbidity of water, sunlight and the presence of predators (tadpoles and fish) also were not associated with the presence of anopheline larvae.

Spatial and temporal distribution of anopheline larval habitats

During the rainy season, most of the water bodies were found within the Pikine department, followed by Rufisque and Guediawaye (Figure 2) but a higher proportion of the water bodies in Rufisque (114/158) and Guediawaye (62/88) contained anopheline larvae as compared to Pikine (399/662) (Table 3). During this season, water bodies encountered in the department of Rufisque were more likely to host anopheline larvae (OR = 1.71, P = 0.006) than the others (Table 3). However during the dry

0.034) where the highest positivity rate for anopheline larval habitats was obtained (26%) (Figure 3).

Molecular identification of *An. gambiae* s.l. species by PCR

All 638 *An. gambiae s.l.* specimens from 13 types of larval habitats across the three departments were identified molecularly as *An. arabiensis* (Figure 4).

DISCUSSION

This study is part of a larger program that seeks to generate data to guide the first LSM in Dakar to further reduce malaria transmission in this area. For this first phase, an area of 107 km² of the 122 km² contained within three of the four departments of the Dakar Region were monitored for one year. This provides a snapshot of the distribution and frequency of anopheline larval sites and allows for planning a potential larval source management intervention.

Our results show that the probability of finding anopheline larval habitats was highest during the rainy season compared to the dry season. Indeed, the daily amounts of rainfall recorded during this period combined with the shallow water table of the study areas caused flooding and consequently long standing water and thus potential malaria vector habitats were created. The presence of stagnant rainwater increased the diversity of anopheline larval sites in rainy season. During this season, anopheline larvae were found in all types of water bodies in all the departments surveyed. Water bodies that had the greatest role in the development of anopheline larvae were ponds, puddles, ravines, drainage channels, streams and canals.

The presence of immature stages of anopheline in puddles has already been reported (Gadiaga et al., 2011; Gillies, 1987; Huang et al., 2006; Miller et al., 2007; Ndenga et al., 2011). For ponds, ravines, drain channels, streams and canals their importance could be explained by the rainfall which reduced the concentration in organics substances allowing the favorable conditions for anopheline larval growth (Chirebvu and Chimbari, 2015; Holstein, 1954). This could explain why in this present study, such a diverse range of habitats were observed. Human mediated factors like poor garbage disposal that subsequently leads to blockage of water flow in drain channels, ravines and streams may also have contributed to the creation of larval habitat similarly that were observed elsewhere (Castro et al., 2010; Keating et al., 2003). Moreover, the absence of predators (tadpoles and fish) in the majority of these types of water bodies made them ideal sites for anopheline immatures.

Most of the water bodies identified and surveyed during the rainy season dried up in the dry season, leading to a significant decrease of potential larval sites as had been
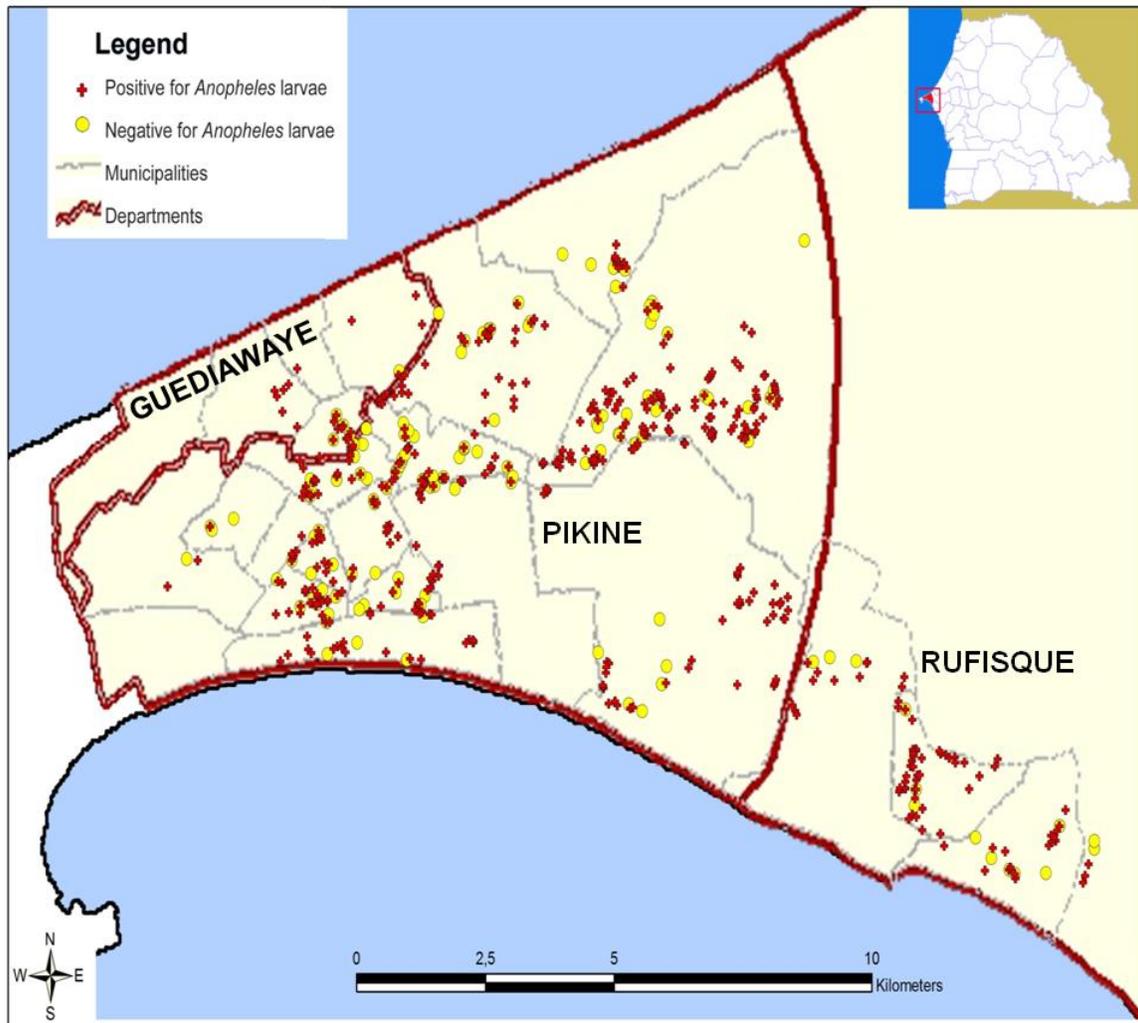

Figure 1. Water turbidity of potential larval habitats: clear (A), more or less turbid (B) and turbid (C) water.

season, anopheline larval habitats were more likely to be found in the Guediawaye department (AOR = 3.15, *P* =

Figure 2. Map of anopheline larval habitats in Dakar Region during the 2013 rainy season. The map highlights the distribution of water bodies in three departments visited among the four in the Dakar region. The water bodies positive for anopheline larvae represented by the red cross and the water bodies negative for anopheline larvae represented by the yellow circle.

shown previously (Simard et al., 2000; Yaro et al., 2012) confirming the importance of the season on the larval habitat distribution. Among the types of water bodies that remained during this period, only the puddles were positively associated with the presence of anopheline larvae. Puddles were created from the resurgence of water from the shallow groundwater table following establishment of a pipeline system.

Although previous studies often reported market-garden wells as the most common anopheline larval habitats (Robert et al., 1998; Trape et al., 1992), only a few were found in the study area. According to Robert et al. (1998) about 5000 market-garden wells were recorded in 1996. Their scarcity nowadays could be a consequence of rapid urbanization (ONU-HABITAT, 2008; RGPHAE, 2013) leading to the disappearance of the majority of the

market-garden wells. However, since this study only surveyed residential areas, we might have missed out other wells that located throughout less populated and rural settings.

During the rainy season, the probability of finding anopheline larvae increases when water bodies were located within 10 m of human habitations. The study sites are located within areas that formerly contained water (marshes, ponds) but dried during the droughts of the 1970s (Faye et al., 1995; Ndao, 2012) and as a consequence the population move in and built houses. However, persistent increases of rainfall amounts are now accompanied by increased flooding in a populated area. This localization of the mosquito larval sites, including anopheline larval habitats near human dwellings brought the oviposition site in the same vicinity of the
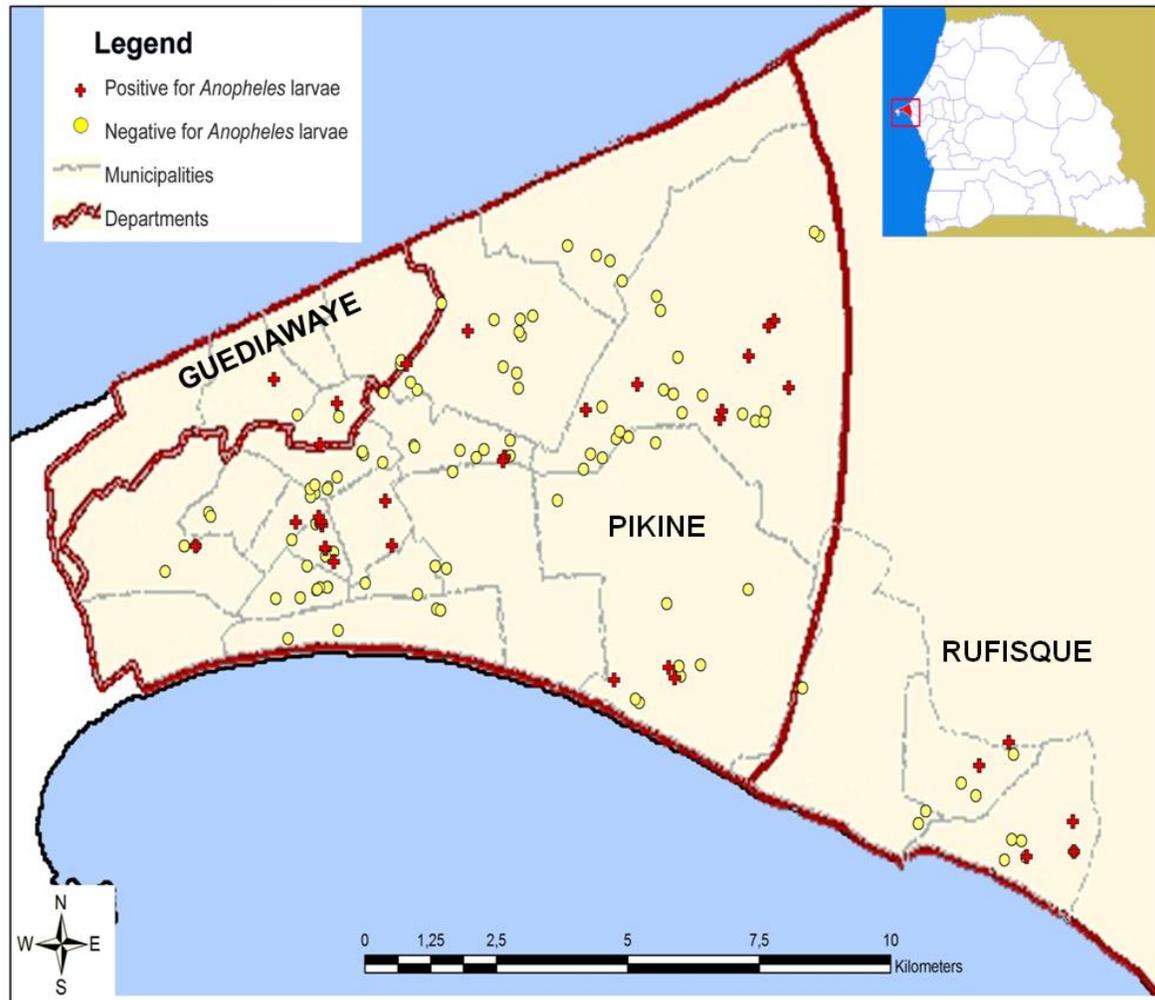
Figure 3. Map of anopheline larval habitats in the Dakar Region during the 2014 dry season. The map highlights the distribution of water bodies in the three departments visited among the four in the Dakar region. The water bodies positive for anopheline larvae represented by the red cross and the water bodies negative for anopheline larvae represented by the yellow circle.

blood meal source, limiting mosquito dispersal (Manga et al., 1993; Njan nloga, 1993) and increasing the risk of malaria transmission (Bogh et al., 2007; Salem, 1994; Thomas and Lindsay, 2000; Trape et al., 1992).

During our survey, anopheline preference for clear water was observed as already described in Sub-Saharan Africa (Bates, 1949; Gimnig et al., 2001; Minakawa et al., 1999; Munga et al., 2005; Mwangangi et al., 2010). However, during the survey, anopheline larvae were also found in the turbid water similarly to a previous study in Ethiopia, where *An. arabiensis* was occasionally found in turbid water (Ye-Ebiyo et al., 2003). Another study in Cameroon, recently reported the development of both *An. coluzzi* and *An. gambiae* in urban polluted waters (Kamdem et al., 2012). The capacity of anopheline vector species to colonize polluted water in urban areas (Awolola et al., 2007; Chinery, 1984; Kamdem et al.,

2012; Sattler et al., 2005) may have an impact on malaria epidemiology within these areas (Keating et al., 2003; Macintyre et al., 2002). Nevertheless, the notion of turbid water may not involve water pollution. Turbidity may be due to materials that can serve as food particles for anopheline and thus contribute to their development (Sattler et al., 2005), but the turbidity may also be caused by matter, such as decaying vegetation, that would impede larval growth (Awolola et al., 2007; Muirhead-Thomson, 1951).

Consistent with previous studies, smaller water bodies identified here were more likely to host *An. gambiae* s.l. larvae (Holstein, 1954; Muirhead-Thomson, 1951; Ndenga et al., 2011), although not consistent with the findings of Majambere et al. (2008) that showed that anophelines were more likely to colonize large water bodies.
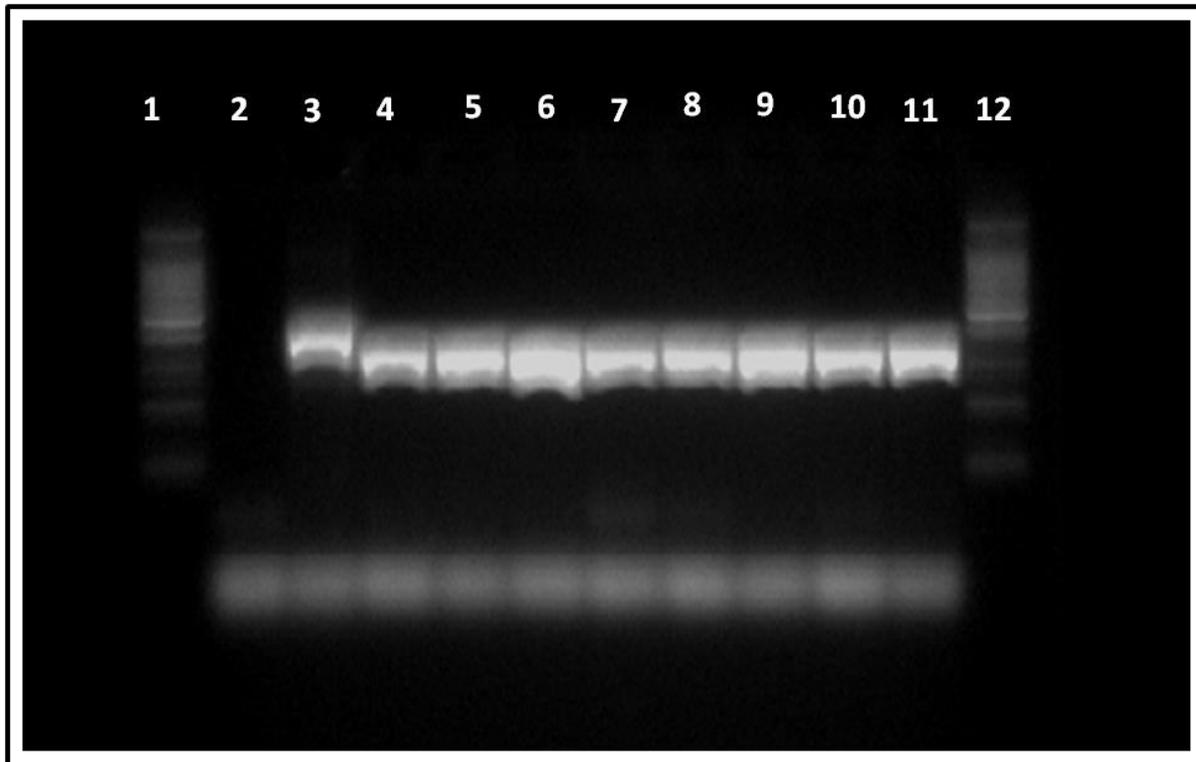
In our study areas anopheline larvae were less

Figure 4. Gel electrophoresis of *An. gambiae* s.l. identification. Lines 1 and 12: 1 kb ladder marker, 2: negative control, 3: positive control (*An. coluzzi*), 4-11: *An. arabiensis*.

frequently encountered in the deep water as previously observed by Clements (Clements, 1992), who explain this observation by the fact that anopheline larvae are surface feeders. However, when in low food conditions at the water surface *An. gambiae s.l.* larvae can increase their diving frequency (Phelan and Roitberg, 2013), which can affect larval mortality as seen in deep water bodies (Tsila et al., 2015; Tuno et al., 2004).

Larvae predators (tadpole and fish) are known to have great impact on mosquito larvae presence and density (Depinay et al., 2004; Gouagna et al., 2012; Kweka et al., 2011; McCrae, 1984; Munga et al., 2005; Munga et al., 2006). This study showed negative association between the presence of tadpoles and anopheline larvae during the rainy season, while no impact was found in the dry season. No association was found between the presence of fish and anopheline larvae in our study. This could be explained by the availability of others food sources for fish in these water bodies (Garcia-Berthou, 1999; Kumar and Hwang, 2006; Specziar, 2004). Thorough studies are needed to assess the role of these larvivorous fish, previously introduced in this area for a biological control (Awono-ambene, 1999). However the presence of these potential larvivorous species may influence the choice of oviposition site of the gravid female in order to safeguard the survival of her offspring (Depinay et al., 2004; Gouagna et al., 2012; Kweka et al., 2011; McCrae, 1984;

Munga et al., 2005; Munga et al., 2006).

The geographical distribution of anopheline larval habitats was heterogeneous across the study areas and the season. During the rainy season, the probability of encountering immature stages of malaria vectors was more important in the Rufisque department. Within this department, the majority of types of water bodies found were those more likely to host anopheline larvae. As explained earlier, most of the anopheline sites were temporary as a most of the sites in Rufisque. In addition, the absence of tadpoles within most of larval sites was often noted in Rufisque department. During the dry season, the scarcity of water bodies was noted and anopheline larvae were associated with the resurgence of groundwater as the result of human activities on shallow water table. Larvae, although found at low numbers, were also observed at the edges of permanent water bodies. *Anopheles arabiensis* was the only member of the *An. gambiae* complex and the only malaria vector that was identified in the study areas and is consistent with previous work within the same region (Awono-Ambene and Robert, 1999; Robert et al., 1998). However, *An. melas* was recently reported in the Niayes area but in very low numbers (Gadiaga et al., 2011).

The Niayes, a coastal marine area, located between inter-dune depressions is characterized by the presence of several ponds and lakes, whose edges are

comparatively clear and sunlit and can act as preferential larval habitats for *An. arabiensis* (Carnevale and Robert, 2009; Rajendran and Reuben, 1991). The predominance of this vector species can also be explained by its high adaptability resulting in colonization of a wide spectrum of water bodies (Pages et al., 2007).

Conclusion

The study showed a wide distribution of anopheline larval habitats across the flooded area of the Dakar suburbs. Although the presence of anopheline larvae in the water bodies does not always mean high larval and adult productivity, abundance of anopheline larval habitats during the period of malaria transmission could influence the production of adult vectors and therefore, the malaria transmission. This study provides baseline database for future implementation of larval source management to accelerate malaria pre-elimination accordingly to the Senegal National Malaria Control Program goals. Further studies are needed to develop effective control strategies against these immature stages of mosquitoes. Regular monitoring of these larval sites would allow evaluate their productivity and to identify periods of risk.

Conflict of interests

The authors declare that they have no conflicts of interest.

Supplemental material

Supp. S1
File format: Word
Title: Types of potential larval habitats.
Description of the main characteristics of the types of potential larval habitats encountered (suburbs of Dakar).

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