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MOSQUITO FAUNA AND PERSPECTIVES FOR INTEGRATED CONTROL OF URBAN VECTOR-MOSQUITO POPULATIONS IN SOUTHERN BENIN (WEST AFRICA)

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Abstract: This study aims at an integrated vector management (IVM) concept of implementing biological control agents against vector mosquito larvae as a cost-effective and scalable control strategy. In the first step, the mosquito species composition fauna of southern Benin was studied using standard entomological procedures in natural and man-made habitats. Altogether, 24 species belonging to 6 genera of mosquitoes *Aedes*, *Anopheles*, *Culex*, *Mansonia*, *Uranotaenia*, *Ficalbia* were recorded. Five species, *Cx. thalassius*, *Cx. nebulosus*, *Cx. perfuscus*, *Cx. pocilipes* and *Fi. mediolineata* are described the first time for Benin. The local mosquito species showed high susceptibility to a *Bacillus sphaericus* formulation (VectoLex® WDG) in a standardized field test. A dosage of 1 g/m^2 was effective to achieve 100% mortality rate for *Cx. quinquefasciatus* late instar larvae in a sewage habitat, with a residual effect of up to 7 days. After more than 1 year of baseline data collection, operational larviciding with *B. thuringiensis* var. *israelensis* and *B. sphaericus* was commenced in 2006 in selected areas. Microbial insecticides products for larval control show great potential within IVM programmes and may augment control efforts against adult insects, such as the use of insecticide-treated bed nets or indoor wall spraying in many parts of Africa.

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INTRODUCTION

At the start of the new millennium, malaria is still deeply entrenched in the tropics, especially in sub-Saharan Africa, and effective malaria control is jeapordized due to lack of effective drugs and insecticide resistance [4, 42]. Therefore, it is imperative that implementation of IMV strategies for malaria vectors and reducing malaria morbidity and mortality using promising microbial control agents, vastly improving environmental sanitation, improved housing, appropriate medical care and intensive public education efforts should be considered as additional tools for curbing the threat of malaria encountered in tropical African countries [43]. Cotonou is the economic capital of Benin. The total population was estimated in 2002 at 780,000 inhabitants on a territory of 73.8 km^2 [17]. It is located on a strip of land between Lake Nokou and the Gulf of Guinea (between latitude 6.2° N–6.3° N and longitude 2.2° E–2.3° E) connected by a lagoon. Environmental conditions enable strong inundations annually in the city, especially at the coastal edges of the lake during the rainy season when the water from the north accumulates and floods low-lying areas of the city. These areas of inundation constitute favourable breeding sites for many mosquito species including

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Anopheles gambiae s.l. Due to high precipitation rates in the region during the rainy season and the structure of the urban area Cotonou has higher levels of malaria transmission (up to 47 infected bites/person/year) compared with other West African cities [1]. The most common malaria strain in Benin is *Plasmodium falciparum* (97.1%) and *P. malariae* (2.9%) [22].

According to the Ministry of Health SNIGS 2003 Annual Report (finalized in October 2004) malaria is the leading cause of mortality among children and leading cause of morbidity in adults in Benin [32]. As in most countries in sub-Saharan Africa (where 85% of all deaths are caused by malaria), malaria impairs economic growth and human development in Benin because the infection rate is 118 of 1,000 adults and 459 of 1,000 for children younger than one year. This cause of death in 2004 was on the level of 11.6%. Malaria remains the primary cause of hospitalization and is responsible for approximately 40% of all health sector consultations among children under 5-years of age.

The malaria control programme in Benin, as well as in other African countries, aims at the reduction of human – vector contact by controlling adult mosquitoes using insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) with pyrethroids and DDT [35]. From the 1950's until the 1990's, DDT was widely used by spraying walls inside the houses, but presently, this method is gradually being phased out. From 2000–2002 a pilot programme campaign was initiated by the Benin health department, referred to as "houses free of mosquitoes". The programme aimed at community participation by educational programmes to train the public in the reduction of breeding sites. It was not successful on the periphery of Cotonou because of the swampy conditions. Since 2000, the major effort to control malaria has been by the distribution of ITNs [23]. Therefore, the implementation of sustainable, environmentally sound and cost-effective alternative strategies to DDT in the mosquito control programme should be realized [20]. The use of microbial agents based on *Bacillus thuringiensis israelensis* (*Bti*) and *B. sphaericus* (*Bs*) in this area appears to be an effective and safe tool to combat malaria in addition to bed nets, residual indoor spraying, and appropriate diagnosis and treatment of malaria parasites as the major tools in the WHO Roll Back Malaria Programme [34]. Vector mosquito larval control may prove to be an effective tool for incorporating into IVM, and may also reduce selection pressure towards resistance to insecticides utilized for ITNs and IRS by reducing the rate of insect-chemical contact in localized populations. In several studies it has been confirmed that larviciding and source reduction have a major advantage in the control of mosquitoes when they are concentrated in their breeding sites, and before they as disperse adults and transmit diseases [10, 19]. A significant feature of *Bti* and *Bs* as biological control agents are their environmental safety. Compared to other pesticides which have a wide range of toxic effects on the environment, these microbial control agents are highly selective in their larvicidal activity and specific targets (mosquito, black fly populations and some nematoceran flies), with no effect on non-target invertebrate or vertebrate organisms [2, 3, 25].

Our study is the first effort to determine mosquito fauna species composition in rural and urban areas of Southern Benin, abundance and phenology (population dynamics) in relation to the climatic conditions, as well as their host preferences. The second aim is to evaluate efficacy of water-dispersible granule VectoLex® WDG formulation based on *Bs* in urban conditions for upcoming large scale trials in Cotonou.

MATERIALS AND METHODS

Entomological studies and classification of mosquito-breeding sites. Investigations on the mosquito species composition, their breeding site preferences, as well as the biting behaviour were carried out in the city of Cotonou, as well as the rural areas of Adjaha-Konho and Grand-Popo from March 2005–May 2007.

All existing and potential breeding habitats in the study areas were categorized and recorded on a map of the study area which was hand drawn and also by using a GPS receiver (Garmin, Model GPS 72). The breeding sites in 100 households were inspected on a weekly basis for larval infestation by dipping. In each of 10–20 dips the number of larval stages L1-L2, L3-L4 and pupae were counted and recorded using a WHO dipper. A sufficient number of larvae were taken to the laboratory for rearing to the 4th instars or adults and detailed species determination [7]. Abiotic data including pH and conductivity were recorded during the study period with combined pH-conductivity measure apparatus (Neolab Art.-No. 4-1028).

Monitoring of adult mosquitoes and biting behaviour. Monitoring of adult mosquitoes was carried out using the Human Bait Catches (HBC) with conventional aspirators in 2 locations in the rural area of Adjaha-Konho. One location was situated in the northern part of the village in a yard covered by clay soil, and the second was in the southern part of the village, close to a structure housing goats, chickens and dogs, between traditional houses. Mosquitoes were collected by one person from 22:00–04:00. All mosquitoes approaching the collector and landing on the person were captured with an aspirator. The mosquitoes were blown into a collecting vessels (PET plastic vessel) which was covered by gauze. A new vessel was used for each hourly collection. The mosquitoes were brought into the laboratory for species determination.

Investigations on host preferences of mosquitoes. To investigate the host preference of the mosquitoes, 4 traps were constructed. Each trap consisted of an interior net (1 × 1 × 1.90 m) and an outer net sized, 2 × 2 × 2 m [3]. The inner net protected the bait from mosquito bites. At each location 2 net traps were placed at a distance of 50 m. In one trap, a human was placed and in the second a goat. The outer net of each trap was opened from about 10 cm from the bottom for 1 hour to allow mosquitoes to approach the host in the inner net; at this time the nets were closed and the collector sampled all mosquitoes in the interior for another hour. The collections were made from 21:00–22:00; 23:00–24:00; 01:00–02:00; 03:00–04:00 during 4 consecutive nights. The mosquitoes were collected by using an exhauster and blown into collecting vessels. The mosquitoes killed in a refrigerator at 5°C in the laboratory, and species determined [7].

Assessment of optimal dosage for biocide based on *Bacillus sphaericus*. In order to assess the optimal dosage for the control of *Cx. quinquefasciatus*, each of 4 cesspools located in Cotonou were treated with VectoLex® WDG (Valent BioSciences Corporation, Illinois, USA) at a dosage rate of 0.1; 1.0 and 5.0 g/m^2. The breeding sites were infested only by *Cx. quinquefasciatus*. The surface treatment area was measured to calculate the exact amount of material for each dosage. Based on the surface area, preweighted amounts of VectoLex® WDG was suspended in 500 ml of water and evenly distributed on the surface with a 1 litre hand-held sprayer. The number of mosquito developmental stages were recorded before treatment, after 24 hours post-treatment and then at 2-day intervals until the mortality rate was lower than 90%. A WHO standard dipper was used and each time 10 dips were taken. For the evaluation of efficacy and residual control, only third and fourth instar larvae were considered because early instars could survive for several days after eclosion and prior to consuming a lethal dose of larvicide. The percentage reduction in larval mosquito densities was calculated using the formula which takes into account the natural changes (for instance through predation) in the mosquito larval populations which take place at the same level and rate in both treated and untreated sites [33].

RESULTS

Species composition of mosquito fauna in southern Benin based on larvae and adult specimens is presented in Table 1. Altogether, 24 species in 6 genera were determinated. Five species *Cx. thalassius*, *Cx. nebulosus*, *Cx. perfuscus*, *Cx. poicilipes* and *Ficalbia mediolineata*, are described for the first time in the *Culicidae* fauna of southern Benin.

Table 1. List of identified species in southern Benin.

Species	Larvae	Adults	First description
Anopheles gambiae s.l. [Giles]		+	
Anopheles pharoensis [Theobald]		+	
Mansonia africanus [Theobald]	+	+	
Mansonia uniformis [Theobald]		+	
Culex antennatus [Becker]	+	+	
Culex bitaeniorhynchus [Giles]	+	+	
Culex duttoni [Theobald]	+		
Culex nebulosus [Theobald]	+	+	+
Culex perfuscus [Edwards]		+	+
Culex poicilipes [Theobald]		+	+
Culex quinquefasciatus [Say]	+	+	
Culex univittatus [Theobald]		+	
Culex thalassius [Theobald]		+	+
Culex tigripes [De Grandpre & De Charmoy]	+		
Culex tritaeniorhynchus [Giles]		+	
Aedes aegypti [Linnaeus]	+	+	
Aedes irritans [Theobald]		+	
Aedes circumluteolus [Theobald]		+	
Aedes luteocephalus [Newstead]	+	+	
Aedes vittatus [Bigot]	+	+	
Uranotaenia annulata [Theobald]		+	
Uranotaenia balfouri [Theobald]	+	+	
Ficalbia mediolineata [Theobald]		+	+
Ficalbia splendens [Theobald]		+	

Types of mosquito-breeding places. During the study period, 8 different types mosquito-breeding places were identified (Tab. 2).

The most abundant breeding places were swamps and water collection jars. In the swampy areas which are characteristic for the region of Grand-Popo, the most abundant species were *An. gambiae s.l.*, *Cx. antennatus* and *Uranotaenia balfouri*. In jars which are used for storage of potable water, a great variety of species were found, such as, *Cx. quinquefasciatus*, *Cx. nebulosus*, *Cx. perfuscus*, *Cx. tigripes*, *Ae. aegypti*, and in smaller quantities *An. gambiae s.l.*, *Cx. duttoni* and *Ae. vittatus*. In rural regions, metal tins, which are usually used as coolers for the distillation of liquor, *Cx. duttoni* and *Cx. tigripes* were abundant. In basins (huge cement containers) used by local farmers in Grand-Popo the following species were found *An. gambiae s.l.*, *Cx. tigripes*, *Cx. perfuscus* and *Ae. vittatus*. Mosquito larvae were also occasionally found in wells (*Cx. quinquefasciatus* and *Cx. tigripes*), plastic containers (*Cx. antennatus*, *Cx. quinquefasciatus*, *Cx. tigripes* and *Ae. aegypti*), plant axis (*Ae. luteocephalus*) and cisterns (*Cx. antennatus*) were also favoured breeding-sites.

Analysis of pH and conductivity showed the widest ranges of both parameters in basins and jars, whereas in the remaining breeding sites more stable life conditions occurred (Tab. 2). The water temperature of all breeding sites ranged from 28.5–31°C.

In the urban area of Cotonou, mosquito larvae were found in the following types of breeding sites: swamps containing dense vegetation, sewage channels, cess pools, temporary water bodies such as puddles, as well as breeding places located inside households, e.g. toilets, utensils (canning jars) and wells. In swampy areas, *An. gambiae s.l.*

Table 2. Abiotic conditions and mosquito larvae species composition in different breeding sites.

Type of breeding site	pH	Conductivity [µS]	Dominant species
Swamps and ponds	7.0–7.8	1.5–2.63	*An. gambiae s.l.*, *Cx. antennatus* and *Ur. balfouri*
Jars	5.9–8.3	0.28–3.95	*Cx. quinquefasciatus*, *Cx. nebulosus*, *Cx. perfuscus*, *Cx. tigripes*, *Ae. aegypti* and in smaller quantities *An. gambiae s.l.*, *Cx. duttoni* and *Ae. vittatus*
Basins	5.3–11.6	0.4–1.5	*An. gambiae s.l.*, *Cx. tigripes*, *Cx. perfuscus*, *Ae. vittatus*
Tins	7.5–8.1	0.7–0.9	*Cx. duttoni*, *Cx. tigripes*
Cisterns	7.6–8.5	0.2–0.4	*Cx. antennatus*
Plastic containers	7.2–8.1	0.3–0.9	*Cx. antennatus*, *Cx. quinquefasciatus*, *Cx. tigripes* and *Ae. aegypti*
Plants	–	–	*Ae. luteocephalus*

and *Cx. antennatus* were the predominant species with average numbers ranging 8–10 larvae/dip and 5–8 larvae/dip, respectively. In cess-pools and sewage channels, the larvae of *Cx. quinquefasciatus* were abundant the most numerous (300 larvae/dip). Most temporary water bodies like puddles located on the streets or in yards, were highly infested with *An. gambiae s.l.* and *Cx. quinquefasciatus* larvae. Characteristic breeding sites for *Ae. aegypti* larvae were various artificial containers as well us coconut shells which occasionally or frequently occurred in almost all households visited.

Biting behaviour of local mosquito populations. During the night, collections made with the HBC at 2 different sites (north and south) in a small village located in the swamp area of Adjaha-Konho (Tab. 3). In the north part, 1,453 adult specimens (69.5%) were collected, whereas in the south part only 638 (30.5%).

Also, hourly observations of mosquitoes' activity (summarized in Tab. 4) show that the highest abundance of mosquitoes were recorded between 23:00–24:00 and 00:00–01:00 (477 and 400 specimens respectively) and the lowest between 01:00–02:00 (262 specimens). In the north part of the village, *Cx. antennatus*, *Ma. uniformis* and *Cx. thalassius* were the most frequently captured species during night time. Females of *Cx. antennatus* and *Cx. thalassius* showed the highest abundance between 23:00–24:00 (6.3% and 5.4%, respectively), whereas *Ma. uniformis* between 03:00–04:00 (4.8%). In the south part of Adjaha-Konho, *Ma. uniformis* was recorded as the most dominant species during the night, whereas after midnight *Ma. africanus* showed also increasing activity (1.8% between 02:00–03:00 and 1.4% between 03:00–04:00 respectively).

Host preferences of the mosquito fauna in the area of Adjaha-Konho. During 4 nights of cage collections baited with 2 different hosts, a total of 6,219 adult specimens out of 6 genera and 14 species were collected (Tab. 5). In general, mosquitoes were frequently found in cages where

Table 3. Number of species collected during the night in village area of Adjaha-Konho.

Species	Place 1 (North part)		Place 2 (South part)	
	number	%	number	%
Ma. uniformis	427	29.4	321	50.3
Ma. africanus	182	12.5	135	21.2
Cx. antennatus	471	32.4	77	12.1
Cx. thalassius	274	18.9	85	13.3
Cx. tritaeniorhynchus	50	3.4	4	0.6
Cx. poicilipes	9	0.6	9	1.4
Culex sp.	3	0.2	0	0
Ae. circumluteolus	12	0.8	3	0.9
Ae. luteocephalus	2	0.1	1	0.2
Ae. agypti	1	0.1	0	0
Aedes sp.	3	0.2	0	0
An. pharoensis	14	1.0	3	0.5
An. gambiae s.l.	1	0.1	0	0
Cx. univittatus	4	0.3	0	0
Total	1,453		638	

Table 5. Host preferences of mosquito fauna in the area of Adjaha-Konho.

Species	Man		Animal	
	number	%	number	%
Ma. uniformis	948	34.6	900	25.9
Ma. africanus	658	24.0	558	16.0
Cx. antennatus	904	33.0	1,558	44.8
Cx. thalassius	93	3.4	195	5.6
Cx. tritaeniorhynchus	59	2.2	101	2.9
Cx. poicilipes	40	1.5	103	3.0
Cx. univittatus	6	0.2	15	0.4
Ae. circumluteolus	9	0.3	11	0.3
Ae. luteocephalus	1	0.0	0	0.0
Aedes sp.	1	0.0	0	0.0
An. pharoensis	20	0.7	31	0.9
An. gambiae s.l.	1	0.0	0	0.0
Ur. annulata	1	0.0	3	0.1
Fi. mediolineata	0	0.0	1	0.0
Fi. splendis	0	0.0	2	0.0
Total	2,741 (44.1%)		3,478 (55.9%)	

Table 4. Night collections of mosquitoes in North (place 1) and South (place 2) part of Adjaha-Konho.

Species	22:00–23:00		23:00–24:00		24:00–01:00		01:00–02:00		02:00–03:00		03:00–04:00	
	place 1	place 2	place 1	place 2	place 1	place 2	place 1	place 2	place 1	place 2	place 1	place 2
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Ma. uniformis	36 (1.7)	51 (2.4)	67 (3.2)	52 (2.5)	65 (3.1)	65 (3.1)	63 (3.0)	35 (1.7)	95 (4.5)	61 (2.9)	101 (4.8)	57 (2.7)
Ma. africanus	19 (0.9)	14 (0.7)	36 (1.7)	13 (0.6)	32 (1.5)	21 (1.0)	35 (1.7)	19 (0.9)	24 (1.2)	38 (1.8)	36 (1.7)	30 (1.4)
Cx. antennatus	58 (2.8)	32 (1.5)	131 (6.3)	23 (1.1)	123 (5.9)	8 (0.4)	60 (2.9)	2 (0.1)	27 (1.3)	3 (0.1)	72 (3.4)	9 (0.4)
Cx. thalassius	32 (1.5)	26 (1.2)	113 (5.4)	15 (0.7)	57 (2.7)	7 (0.3)	16 (0.8)	10 (0.5)	16 (0.8)	12 (0.6)	40 (1.9)	15 (0.7)
Cx. tritaeniorhynchus	1 (0.1)	2 (0.1)	8 (0.4)	2 (0.1)	10 (0.5)	0	14 (0.7)	0	11 (0.5)	0	6 (0.3)	0
Cx. poicilipes	0	0	1 (0.01)	2 (0.1)	3 (0.1)	2 (0.1)	0	2 (0.1)	2 (0.1)	3 (0.1)	3 (0.1)	0
Culex sp.	0	0	3 (0.1)	0	0	0	0	0	0	0	0	0
Ae. circumluteolus	6 (0.3)	2 (0.1)	2 (0.1)	1 (0.1)	1 (0.1)	0	0	0	0	0	3 (0.1)	0
Ae. luteocephalus	0	0	2 (0.1)	0	0	0	0	0	0	0	0	1 (0.1)
Ae. agypti	0	0	0	0	0	0	0	0	0	0	1 (0.1)	0
Aedes sp.	0	0	1 (0.1)	0	2 (0.1)	0	0	0	0	0	0	0
An. pharoensis	3 (0.1)	2 (0.1)	5 (0.2)	0	3 (0.1)	0	1 (0.1)	1 (0.1)	2 (0.1)	0	0	0
An. gambiae s.l.	0	0	0	0	1 (0.1)	0	0	0	0	0	0	0
Cx. univittatus	0	0	0	0	0	0	4 (0.2)	0	0	0	0	0
Total number of specimens (%)	**289**		**477**		**400**		**262**		**294**		**374**	
	155 (7.4)	129 (6.2)	369 (17.6)	108 (5.2)	297 (14.2)	103 (4.9)	193 (9.2)	69 (3.3)	177 (8.5)	117 (5.6)	262 (12.5)	112 (5.4)

animals were used as bait (55.9%), and the most abundant species was *Cx. antennatus* (44.8%) followed by *Ma. uniformis* (25.9%) and *Ma. africanus* (16.0%). *Aedes circumluteolus* and *An. pharoensis* were rarely found (0.3% and 0.9%, respectively). In cages with human bait, *Ma. uniformis*, *Cx. antennatus* and *Ma. africanus* were frequently recorded but at different rates (34.6%, 33.0%, 24.0%, respectively). *Anopheles pharoensis* and *An. gambiae s.l.* were rarely recorded (less than 1% of abundance).

Assessment of the optimal dosage for biocide based on *Bacillus sphaericus*. The results of the efficacy test formulation of VectoLex® WDG against third and fourth instar of *Cx. quinquefasciatus* larvae are summarized in Table 6. The lowest dosage (0.1 g/m^2) was not sufficient to reduce the larval population to an acceptable level. One day after application the larval mortality rate was lower than 30%. The application of 1 g/m^2 resulted in 100% mortality on the first day after application. On day 3, the mortality rate was lower (85.5%). The highest dosage of 5 g/m^2 resulted in a sufficient mortality rate (above 99%) for 7 days. On day 16th, the mortality rate decreased below 90% and showed only 81.0%.

At the test sites, the water was polluted and the results of water quality parameters were: pH range between 7.1–7.8; conductivity range between 1.4–1.5 µS and water temperature between 28–31ºC.

DISCUSSION

Our study showed that 24 mosquito species were present in the rural and urban area in Cotonou, including species which have been described for the first time for Benin (*Cx. thalassius*, *Cx. nebulosus*, *Cx. perfuscus*, *Cx. poicilipes*). The larvae of these species were found in different natural and man-made breeding sites. The most popular were basins, plastic containers and jars. Clay jars play an important role in West-African culture. The bigger ones with

Table 6. Absolute numbers and calculated mortality of larval stages L3-L4 after application of different VectoLex® WDG dosages.

Dosage [g/m^2]	Average number of larvae						Mortality (%)				
	Day						Day				
	Pre*	1	3	5	7	16	1	3	5	7	16
Control	126.0	91.0	120.0	318.0	176.0	117.0					
0.1	69.7	38.0	31.7	–	–	–	26.2	53.2	–	–	–
1.0	32.5	0.00	4.5	2.8	–	–	100.0	85.5	–	–	–
5.0	92.0	0.00	0.00	0.5	0.00	17.0	100.0	100.0	99.8	100.0	81.0

*Pre-number of larvae before application, 1–16 – number of days after application.

a volume of 30–60 liters are called "Jarres", and are used for fresh water storing. Smaller jars, called "Cannaries" are used for many purposes, like drinking troughs for animals and for religious reasons. In rural regions, metal tins are used as coolers for the distillation of liquor, an important commodity of the local trade. If the tins are not in use the water is left there and they serve as good breeding places for *Cx. duttoni* and *Cx. tigripes*.

In our study, the most abundant genus *Culex* was represented by 10 species. In the rural areas, *Cx. antennatu*s and *Cx. thalassius*, were the most abundant. Larvae of *Cx. thalassius* breed in a wide variety of sun exposed habitats with stagnant and clean water like crab holes, wells, ditches and small metal tins [15, 16]. *Cx. thalassius* is widely distributed in Africa, especially in the coastal part [18]. The peak biting activity of females is after twilight; however, they can be troublesome during the night and also at dawn. In our study, 2 peaks of biting activity in the north part of Adjaha-Konho were recorded as follows: after dusk (23:00–24:00) and during the night (03:00–04:00). The females were frequently recorded in the cages baited with animal hosts.

Larvae of *Cx. antennatus* breed in clean as well as stagnant water bodies. In our study, the larvae were found in swampy areas, cisterns and plastic containers with fresh water. The females are considered to be strongly zoophilic feeding mainly on livestock [5, 14]. In our study, the peak of adult female activity in the north part of Adjaha-Konho was recorded between 22:00–24:00. Also in the north part of this village during bite behaviour research *Cx. antennatus* was the most abundant species in cages with an animal host (44.8%), and the second in order of appearance in cages where man was used as bait (33.0%). Differences in results of both research between north and south part of Adjaha-Konho can be explained by the lack of competitive baits close to the net traps.

Larvae of *Cx. nebulosus* prefer man-made water bodies like construction sites, plastic containers, water barrels, and utensils, but also in tree holes [15]. Females feed mostly on human blood [18]. In Grand-Popo, larvae were found in canarries in dark breeding water which showed a high value of conductivity. Larvae of *Cx. nebulosus* may occur in the same habitats as *Cx. quinquefasciatus*, but were displaced by this species [37]. Larvae of *Cx. perfuscus* can be found both in forest temporary pools, streams or swamps which usually have a considerable amount of organic matter and slight vegetation, as well as in other artificial containers like water barrels or utensils [15]. In our study, larvae of this species frequently occurred in basins and jars; nevertheless, adult specimens were not recorded during night collections. The peak activity of adult *Cx. poicilipes* begins after dusk and gradually increases until 03:00, whereas in our study, the females of *Cx. poicilipes* were mostly recorded during the night [13]. The females of *Cx. poicilipes* preferably feed on animals, this was also confirmed in our study in Adjaha-Konho village.

Females of *Cx. quinquefasciatus* are known for indoor and outdoor antropophilic biting activity during the whole night [14]. The all night long activity of adult females was observed in the Grand-Popo catch study. In Adjaha-Konho during a period of drought, a lot of potential breeding sites of *Cx. quinquefasciatus* dried out, resulting in the lack of larvae and adult specimens during the study period. In the study area of Cotonou, numerous larvae of *Cx. quinquefasciatus* (300 larvae/dip) were found, mostly in sewage channels. This species has to be carefully controlled during routine programmes to achieve a better acceptance by the people. It is mainly *Cx. quinquefasciatus* which people recognize as mosquito nuisance. If the control would focus only on anophelines, people would not recognize the positive effect of the control efforts for the life quality of their lives.

In our study, the genus *Anopheles* was represented by only 2 species, *An. gambiae s.l.* and *An. pharoensis*, whereas for Benin 22 different species of *Anopheles* are described [36]. Because of the exceptional drought in the year 2005, in village area larvae of *Anopheles* sp. were not numerous. In Cotonou, many of the temporary water bodies, such as puddles located on the streets or in the yards, were highly inhabited by *An. gambiae s.l.* In the costal area, as well as in the whole country, the 2 most important vectors for malaria parasites *An. gambiae s.s.* and *An. melas* are abundantly present [36]. Both species are members of the Anopheles Gambiae Complex, the similar look-alike species are distinguishable from each other by their behaviour. *An. melas* tolerates high salinity in water used as breeding-sites, whereas *An. gambiae s.s.* is more reliant on fresh water. The different behaviour of the vectors influences the chronology and intensity of malaria cases [1]. In the city of Cotonou, the epidemiological situation is seasonal, with the peak of malaria infection correlating with the rainy seasons (May–June and August–December) which creates fresh water sources, the preferred breeding sites of *An. gambiae s.s.*

Host preferences as well as hourly observations of mosquito fauna in the area of Adjaha-Konho showed that *Ma. africana* and *Ma. uniformis* were the predominant species. Adult females of *Ma. africana* predominantly feed on humans but they may also feed on other mammals, mostly monkeys [12, 27, 38]. Field experiments using cage collections baited with 2 different hosts showed that *Ma. africana* was more frequently found in cages where human bait was located in comparison to cages with bovine hosts [39]. To feed on humans, the females readily enter dwellings during dusk with the maximum peak 2 hours after dusk, at midnight and during the night [13, 18, 31]. They may also migrate for moderate distances, up to 10 km from the breeding sites [40]. *Ma. uniformis* is a species widely distributed in the sub-Saharan part of Africa, as well as in India and in Southeast Asia [26]. In our study, the highest activity of *Ma. africana* females in both studied areas was recorded after 02:00, but other authors observed increased activity during twilight or throughout the night [13, 18].

Precise knowledge of the relative abundance and phenology of the relevant mosquito species is essential to fulfill the requirements of an economical and ecologically successful control programme of vector mosquito populations with a special regard for the biological control agent based on *Bti* and *Bs*. The most effective method for controlling vector populations is to control the larvae at their breeding sources before they emerge as adults. Larval control of mosquitoes has major advantages over adult control. The phenomenon of this issue can be explain that in contrast to adults, larvae are concentrated in predictable sites that can be easily accessed, treated, or manipulated with no chance of the larvae escaping. High density of vector populations can be killed when they are condensed in a very limited area with less input into the environment.

Bti and *Bs* are environmentally-friendly control agents, targeted specifically against the malaria vector-species with minimal impact on non-target organisms and the environment, allowing extensive and intensive use while keeping the ecosystem functionality and services unharmed [29, 30]. When applied in an organized programme *Bti* and *Bs* have fully suppressed adult vector-mosquito emergence and substantially reduced entomological inoculation rates [8, 10].

Microbial larval control of malaria vector-mosquitoes therefore has great potential within IVM programmes and may augment control efforts against adult vector stages, such as the use of ITNs and IRS, in many parts of Africa [6, 8, 10, 11, 19, 20, 21, 24, 28, 41, 44, 46]. Larval control strategies have also historically contributed to successful reductions of malaria transmission [21, 41]. Such strategies could provide highly effective malaria-vector control methods, complementary to adult control interventions, and should be prioritized for further development, evaluation and implementation as an integral part of Roll Back Malaria [21]. This strategy is also cost-effective in relation to distribution of impregnated bed-nets [8]. The assessed cost/person protected by larval control under various African scenarios range from 0.90–2.50 US Dollars, correlating negatively with human population density [10, 45]. Both estimates seem rather conservative because they assume the need for weekly applications of larvicides, at a high rate, to all the breeding sites. During our preliminary study, it was estimated that in a district of Cotonou with 1,000 houses, less than 1 ha of breeding sites had to be controlled to eliminate the anophelinae larvae (less than 500 grams of the products are needed = amounting to less than 15 US Dollars/district/per treatment/control phase). This opens up the possibility of a successful and cost-effective control of many vector species in Cotonou. Since 2006, a community-based, large-scale programme of routine microbial control agents application has been conducted in Arrondissement No. 6 of Cotonou. In preparation phases of preliminary studies, it could be shown that the major breeding sites are small ponds (mass breeding sites of *An. gambiae s.l.*) mainly in yards of the houses, swamps mainly along the shore of the Lac Nokoue (e.g. Ladji), channels along the streets and sewage tanks. The last 2 types are mass breeding sites of *Cx. quinquefasciatus* which is the most important vector of lymphatic filariasis. Precise mapping and individual numbering of each significant breeding site is important for precise communication between field staff. All houses on each site of the streets were numbered (right site of the streets = odd; e.g. 1, 3, 5 etc., and the left site of the streets even numbers, e.g. 2, 4, 6 etc.) and mapped. This study is still in progress and the data compared with the control area will provide a solid basis for future routine operations.

In several East-African countries, such as Kenya and Tanzania, sufficient results using *Bti* and *Bs* formulations have been achieved [8, 9, 10]. The following formulations and dosages have been used in a pilot project in Mbita, Lake Victoria, Kenya: VectoBac® WDG (3,000 ITU/mg); VectoBac® G (200 ITU/mg); VectoLex® WDG (650 ITU/mg); VectoLex® CG (50 ITU/mg). The optimum effective dosages were assessed as 0.2 kg/ha for WDGs, 4 kg/ha for VectoBac® G and 2 kg/ha for VectoLex® CG. The larvae of *An. gambiae s.s.* proved to be more susceptible to *Bs* than to *Bti*. In Cotonou, environmentally friendly bio-control agents will be also integrated with physical manipulation of the mosquito producing habitats by alteration of temporary pools by drainage wherever possible or by filling-in, as well as propagation and utilizing natural enemies (*Gambusia* fish or preferably indigenous fish species). This study should lead in the future to the implementation of bio-control agents application under prevailing conditions (mapping and routine use of *Bti* and *Bs*) in an integrated control programme, including entomological, parasitological and epidemiological studies in the control, and in a comparable check area, as well as to train personnel in the Health Administration in Cotonou. Public health surveillance of malariometric parameters will serve as a measure of the success of control measures taken under the framework of this malaria control strategy. In the final analysis, the integrated biological control of mosquito larvae by means of *Bti*/*Bs* and physical alteration of breeding sites will result in considerable benefits to environmental quality and public health, and contribute to the country's general economy.

CONCLUSION

Environmentally safe microbial larvicides can significantly reduce larval abundance in the natural and man-made habitats and could be a useful tool for inclusion in an IVM programme. This novel management, based on a monitoring and evaluation system for implementing routine larviciding of mosquito vectors in African cities, has shown considerable potential for sustained, rapidly responsive, data-driven and affordable application.

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REFERENCES

1. Akogbeto M, Chippaux JP, Coluzzi M: Le paludisme urbain côtierà Cotonou (République du Bénin): étude entomologique. *Rev Epidemiol Sante Publique* 1992, **40**, 233–239.

2. Becker N: Microbial control of mosquitoes: management of the Upper Rhine mosquito population as a model programme. *Parasitol Today* 1997, **13**, 485–487.

3. Becker N, Petric D, Zgomba M, Boase C, Dahl C, Lane J, Kaiser A: *Mosquitoes and their Control*. Kluwer Academic Publishers, New York, London 1997.

4. Coleman M, Sharp B, Seocharan I, Hemingway J: Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *J Med Entomol* 2006, **43**, 663–668.

5. Costantini C, Sagnon NF, della Torre A, Diallo M, Brady J, Gibson G, Coluzzi M: Outdor-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. *Am J Trop Med Hyg* 1998, **58**, 56–63.

6. Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H, Fillinger U, Drescher AW, Tanner M, Castro MC, Killeen GF: Participatory mapping of target areas to enable operational larval source management to suppress malaria vector mosquitoes in Dar es Salaam, Tanzania. *Am J Trop Med Hyg* 2007, **77**, 74–74.

7. Edwards FW: *Mosquitoes of the Ethiopian Region. III. – Culicine Adults and Pupae*. British Museum (Natural History), London 1941.

8. Fillinger U, Knols BGJ, Becker N. Efficacy and Efficiency of New *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* Formulations Against Afrotropical Anophelines in Western Kenya. *Trop Med Int Health* 2003, **8**, 37–47.

9. Fillinger U, Sonye G, Killeen GF, Knols BGJ, Becker N: The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: operational observations from a rural town in western Kenya. *Trop Med Int Health* 2004, **9**, 1274–1289.

10. Fillinger U, Lindsay SW: Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Trop Med Int Health* 2006, **11**, 1629–1642.

11. Fillinger U, Kannady K, William G, Vanek MJ, Dongus S, Nyika D, Geissbuehler Y, Chaki PP, Govella NJ, Mathenge EM, Singer BH, Mshinda H, Lindsay SW, Tanner M, Mtasiwa D, de Castro MC, Killeen GF: A tool box for operational mosquito larval control: preliminary results and early lessons from the urban malaria control programme in Dar es Salaam, Tanzania. *Malar J* 2008, **7**, 1–20.

12. Haddow A, Dick G: Catches of biting Diptera in Uganda, with anaesthetized monkeys as bait. *Ann Trop Med Parasitol* 1948, **42**, 271–277.

13. Hamon J: Les moustiques anthropophiles de la région de Bobo-Dioulasso (République de Haute-Volta): Cycles d'agressivité et variations saisonnières. *Ann Soc Ent France* 1963, **132**, 85–144.

14. Hamon J, Sales S, Coz J, Ouedraogo C, Dyemkouma A, Diallo B: Observations sur les préférences alimentaires des moustiques de la République de Haute-Volta. *Bull Soc Path Exot* 1964, **57**, 1133–1150.

15. Hopkins G: *Mosquitoes of the Ethiopian Region I. – Larval Bionomics of Mosquitoes and Taxonomy of Culicine Larvae with Notes and Addenda* by P. F. Mattingly. British Museum (Natural History), London, 1952.

16. Ingram A, Macfie JWS: The early stages of certain West African mosquitoes. *Bull Ent Res* 1917, **8**, 135–154.

17. Institut National de la Statistique et de l'Analyse Economique (INSAE): *Recensement général de la population et de l'habitation, synthčse des résultats d'analyse*. Cotonou, Bénin (RGPH3), Cotonou 2003.

18. Kerr J: Studies on the abundance, distribution and feeding habits of some West African mosquitoes. *Bull Ent Res* 1933, **24**, 493–510.

19. Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF, Beier JC: The potential impact of integrated malaria transmission control on entomologic inoculation rate in highly endemic areas. *Am J Trop Med Hyg* 2000, **62**, 545–551.

20. Killeen GF, Fillinger U, Knols BGJ: Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malar J* 2002, **1**, 1–7.

21. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ: Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *Lancet Infect Dis* 2002, **2**, 618–627.

22. Kinde-Gazard D, Gbenou D, Tohon S, da Silva C, Nahum A, Quenum A, Houndigande E, Houndekon R, Ekoue S, Massougbodji A: Monitoring and assessment indicators in 2001 of „Roll Back Malaria" initiative in Benin. *Bull Soc Pathol Exot* 2004, **97**, 349–352.

23. Kinde-Gazard D, Gbenou D, Houngnihin R: Community based actions for fighting against malaria. **In:** Koning LO (Ed): *Progress in malaria research*, 211-216. Nova Science Publishers Inc., 2007.

24. Kroeger A, Horstick O, Riedl C, Kaiser A, Becker N: The potential for malaria control with the biological larvicide *Bacillus thuringiensis israelensis* (*Bti*) in Peru and Ecuador. *Acta Trop* 1995, **60**, 47–57.

25. Lacey L: *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *J Am Mosq Control Assoc* 2007, **23**, 133–163.

26. Laurence B: Oviposition by mansonioides mosquitoes in the Gambia, West Africa. *Proc R Ent Soc Lond* 1959, **34**, 161–170.

27. Lee VH, Monath TP, Tomori O, Fagbami A, Wilson DC: Arbovirus studies in Nupeko forest, a possible natural focus of yellow fever virus in Nigeria. II. Entomological investigations and viruses isolated. *Trans R Soc Trop Med Hyg* 1974, **68**, 39–43.

28. Majambere, S, Lindsay SW, Green C, Kandeh B, Fillinger U: Microbial larvicides for malaria control in The Gambia. *Malar J* 2007, **6**, 76.

29. Margalith Y: Biological control by *Bacillus thuringiensis* subsp. *israelensis* (*Bti*); history and present status. *Israel J Entomol* 1989, **23**, 3–8.

30. Margalith Y: The discovery of *Bacillus thuringiensis israelensis*. **In:** de Barjac H, Sutherland DJ (Eds): *Bacterial control of mosquitoes and Black Flies*, 3-9. Rutgers University Press, New Brunswick, NJ 1990.

31. Mattingly P: Studies on West African forest mosquitoes, Part I – The seasonal distribution, biting cycle and vertical distribution of four of the principal species. *Bull Ent Res* 1949, **40**, 149–168.

32. Ministry of Health: *Annual Report*, 2003. SNIGS, Benin 2004.

33. Mulla MS, Norland RL, Fanara DM, Darwazeh A, McKean D: Control of chironomid midges in recreational lakes. *J Econ Entomol* 1971, **64**, 300–307.

34. Nabarro DN, Tayler EM: The Roll Back Malaria campaign. *Science* 1998, **280**, 2067–2068.

35. Roberts DR, Manguin S, Mouchet J: DDT house spraying and reemerging malaria. *Lancet* 2000, **356**, 330–332.

36. Scott J, Brogdo W, Collins F: Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg* 1993, **49**, 520–529.

37. Service MW: The replacement of *Culex nebulosus* Theo. by *Culex pipiens fatigans* Wied. (Diptera, Culicidae) in towns in Nigeria. *Bull Entomol Res* 1966, **56**, 407–415.

38. Smith A: On the transmission of bancroftial filariasis on Ukara Island, Tanganyika. IV. Host-preferences of mosquitoes and the incrimination of *Anopheles gambiae* Giles and *An. funestus* Giles as vectors of bancroftial filariasis. *Bull Ent Res* 1955, **46**, 505–515.

39. Snow WF: The attractiveness of some birds and mammals for mosquitoes in The Gambia, West Africa. *Ann Trop Med Parasitol* 1983, **77**, 641–651.

40. Vincke I: Note sur les Culicidés de la vallée de la Lufira (1941–1942). *Riv Parasit* 1959, **20**, 423–433.

41. Walker K, Lynch M: Contributions of *Anopheles* larval control to malaria suppression in tropical Africa: review of achievements and potential. *Med Vet Entomol* 2007, 21, 2–21.
42. Winstanley PA, Ward SA, Snow RW: Clinical status and implications of antimalarial drug resistance. *Microbes Infect* 2002, **4**, 157–164.
43. WHO: *Guidelines for integrated vector management*. Harare, WHO Regional Office for Africa, 2003.
44. WHO: *Partager les experiences et echanger les connaissances sur la demoustication pour ameliorer la lutte contre le Paludisme au Benin*. World Health Organization, Conference, Cotonou, Benin, 2008.
45. Worrall E: *Integrated vector management programs for malaria control – cost analysis for large-scale use of larval source management in malaria control*. Prepared for: Bureau for Global Health, United States Agency for International Development (USAID) 2007, Contract GHS-I-01-03-00028-000-1.
46. Yohannes M, Haile M, Ghebreyesus TA, Witten KH, Getachew A, Byass P, Lindsay SW: Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? *Trop Med Int Health* 2005, **10**, 1274–1285.