Spatio-temporal variations of *Anopheles coluzzii* and *An. gambiae* and their *Plasmodium* infectivity rates in Lobito, Angola

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ABSTRACT: From 2003 to 2007, entomological surveys were conducted in Lobito town (Benguela Province, Angola) to determine which *Anopheles* species were present and to identify the vectors responsible for malaria transmission in areas where workers of the Sonamet Company live. Two types of surveys were conducted: (1) time and space surveys in the low and upper parts of Lobito during the rainy and dry periods; (2) a two-year longitudinal study in Sonamet workers' houses provided with long-lasting insecticide-treated nets (LLIN), "PermaNet," along with the neighboring community. Both species, *An. coluzzii* (M molecular form) and *An. gambiae* (S molecular form), were collected. *Anopheles coluzzii* was predominant during the dry season in the low part of Lobito where larvae develop in natural ponds and temporary pools. However, during the rainy season, *An. gambiae* was found in higher proportions in the upper part of the town where larvae were collected in domestic water tanks built near houses. *Anopheles melas* and *An. listeri* were captured in higher numbers during the dry season and in the low part of Lobito where larvae develop in stagnant brackish water pools. The infectivity rates of *An. gambiae* s.l. varied from 0.90% to 3.41%. ***Journal of Vector Ecology* 40 (1): 172-179. 2015.**

Keyword Index: *Anopheles coluzzii*, *Anopheles gambiae,* malaria, infectivity, Angola.

INTRODUCTION

One of the most effective ways to reduce the burden of malaria is to control the *Anopheles* mosquitoes that transmit the pathogenic agents (Neafsey et al. 2010), but well targeted and effective control can only be planned and achieved if malaria vectors are accurately identified and their specific bionomics, role in malaria transmission, and geographic distribution are correctly understood.

Entomological studies have long been conducted in Angola (Roque 1903, Giles 1904, Wellman 1905, Correia Mendes in Sant'Anna 1920, Colaço 1952, Gândara 1956). Large entomological studies were conducted by Ribeiro and colleagues before the beginning of the civil war of November, 1975) (Ribeiro 1966, 1969), including a major entomological survey at 147 sites in Angola (Ribeiro and Ramos da Cunha 1975). Although, the 27 years of civil war drastically limited further study on malaria until its end in 2002, Ribeiro and Ramos da Cunha (1995) did record some 230 species and subspecies of mosquitoes in Angola, among which were 47 *Anopheles* species. Two main malaria vectors, *An. gambiae* s.l. and *An. funestus*, were reported, as well as *An. melas*, a brackish water vector present along the western coastal areas from Mauritania to Angola (Sinka et al. 2010). More recent surveys in the country have confirmed the presence of *An. funestus,* some *An. arabiensis*, and both M and S molecular forms of *An. gambiae* s.l. (Calzetta et al. 2008, Carrara et al. 2002, Cuamba et al. 2006, Santolamazza et al. 2008, Toto et al. 2011), now formally designated as *An. coluzzii* and *An. gambiae,* respectively (Coetzee et al. 2013). We used the terms of *An. gambiae* for the S form only, while *An. gambiae* s.l. refers to the complex of species, *An. coluzzii* and *An. gambiae*. These studies showed the dominance of *An. coluzzii* in the tropical dry, semi-arid, and anthropized environments of some coastal provinces of Angola (Luanda, Benguela, Namibe), while *An. gambiae* was highly predominant in tropical humid highlands and the rural areas of coastal provinces (Cabinda, Zaire) and inland (Cuanza Norte, Malanje, Lunda Sul, Huambo), yet was absent from areas studied in Luanda and Namibe. Several specimens of *An. melas* were also found in the same sites on the Luanda coast as *An. coluzzii*. Only one specimen of *An. arabiensis* was found in the study sites in Luanda, Huambo and Namibe Provinces. The *Plasmodium falciparum* sporozoite rate for *An. gambiae* s.l. ranged from 0.4% (Carrara et al. 2002) to 1.9% (Cuamba et al. 2006), with infection rates of 0.4% for *An. coluzzii* and 3.7% for *An. gambiae* (Calzetta et al. 2008), while *An. funestus* had a sporozoite rate of 0.7% (Cuamba et al. 2006). In their survey of April, 2001 by indoor pyrethrum spray, Cuamba et al. (2006) found that *An. coluzzii* was predominant, representing over 93%. *Anopheles gambiae* was found only in limited coastal areas, and commonly in the humid highlands of Huambo but was not recorded in Lobito. Insecticide resistance studies were performed (Santolamazza et al. 2008, Toto et al. 2011), and after classical WHO bioassays tests done in June, 2003, March, 2004,

and November, 2005 with specimens collected in Lobito from Alto Liro (*An. coluzzii* and *An. gambiae*), Sao Joao (*An. coluzzii* and *An. gambiae*), and Bela Vista (only *An. gambiae*) Districts, Toto et al. (2011) considered that "the main local malaria vector *An. gambiae* s.l. (both *An. coluzzii* and *An. gambiae*) was basically resistant to DDT and susceptible to all pyrethroids, regardless of the period and the site of collections." Such observations justify the implementation of deltamethrin-impregnated nets. Further tests done in December, 2012 with *An. gambiae* s.l. collected in the "Yard part" of Lobito (low level of the city with a predominance of *An. coluzzii*) showed 100% mortality with diagnostic dosage of permethrin (at 0.75%) and 92% with deltamethrin (at 0.05%) (Toto et al., unpublished).

Our study was motivated by the concern of the Medical Department of the Sonamet Company (an Angolan construction company for offshore projects) to reduce confirmed malaria cases among employees living in suburbs of Lobito. As relatively little was known about the malaria vectors and their behavior in Lobito, entomological surveys were needed to identify and monitor the *Anopheles* species acting as malaria vectors in areas where workers were living. Our goal was also to identify the vector breeding sites as part of an integrated malaria prevention program including larval control activities (conducted by the National Malaria Control Programme), free of charge distribution of Permanet® 2.0, a long-lasting insecticidal net (LLIN), to the families of Sonamet employees, and delivery of an information-education-communication (IEC) campaign to reinforce correct use of the prevention tools and encourage early and appropriate treatment-seeking behavior.

Evaluation of this integrated malaria prevention program was based upon entomological, parasitological, and newly developed immunological parameters related to the development of antibodies against the salivary protein of mosquitoes found in the blood of inhabitants (Drame et al. 2010a, Drame et al. 2010b).

MATERIALS AND METHODS

Study area

Surveys were conducted in Lobito (12°19'S; 13°34'E), a town of more than 200,000 inhabitants located on the Atlantic seashore in Benguela Province, 400 km south of Luanda, the capital of Angola. Lobito is built on the Central Plateau with a temperate tropical climate, which normally includes a dry season (May-October) and a rainy season (November-April) with 200 to 1,000 mm of annual rainfall. The average temperature varies between 20° C to 27° C and the average relative humidity is around 80%.

Lobito can be schematically divided into two main areas (Figure 1); the low part, which is located at sea level, and the upper part where houses are built on a cliff 70-100 m above sea level. In the low part, large areas of standing brackish surface water, often fed by seawater during high tide, provide a suitable breeding habitat for *Anopheles melas* and *An. listeri* (Gillies and de Meillon 1968) (Figure 2A). In the upper part, *Anopheles* larval sites were mainly limited to human-made cement storage water tanks designed for domestic usage and built near houses (Figure 2B).

All malaria cases diagnosed by medical doctors of the Sonamet's medical department were mapped using GPS localization techniques and integrated into a GIS. Case mapping clearly identified some "clusters" of malaria in different parts of the town. Our surveys targeted these hot spots of malaria to generate data on vectors and malaria risk, and to monitor impact of Sonamet's prevention activities.

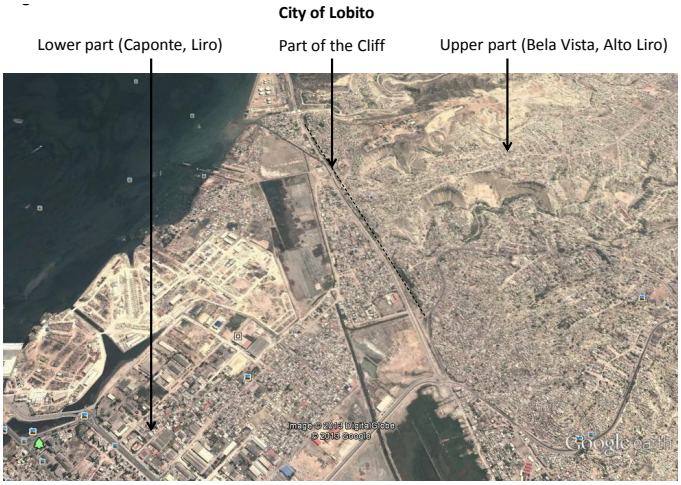

Figure 1. Map of Lobito showing the lower part (left) and upper part (right) of the town divided by a cliff (dotted line) (source: Google Earth, 2013).

Entomological methods

Two protocols were followed: (1) four spatio-temporal mosquito surveys, designed to identify the mosquito species present in the lower and upper parts of Lobito during the dry and rainy periods, were implemented between 2003 and 2007; (2) a two-year longitudinal survey was implemented in 2005-2006, both years from January to December, to evaluate the impact of free distribution of long-lasting insecticide-treated nets (LLIN, Permanet 2.0®) to Sonamet workers and their families.

The aim of these time and space surveys was to clearly identify the main *Anopheles* mosquito species present in areas where transmission was occurring, confirm which were malaria vectors, and identify their larval habitats to help target malaria control activities. In order to increase the diversity of adult mosquito species sampled, several methods were used, including CDC light traps (CDC LT) that were used indoors from 18:00 to 06:00, indoor human landing collections (HLC) from 18:00 to 06:00, and morning indoor residual resting fauna (IRF) using a mouth aspirator. In addition, larval collections were made by classical dipping in all natural and anthropic breeding sites found in the hot spots. Sampling efforts were equivalent in the lower and upper parts of Lobito and also during the different years. The surveys were conducted during the dry seasons of 2003 and 2007 and the rainy seasons of 2004 and 2005. It is important to note that 2003, 2004, and 2005 experienced normal seasonal patterns; however, 2007 suffered an unusually short rainy season, limited to March and April. Adult mosquitoes were collected in an average of 24 sites per survey with an equal number of collecting sites in the lower part (at sea level) in the districts of Restinga, Lobito Velho, Sonamet-Yard, Caponte, Liro, and Sao Joao, and in the upper part in the districts of Bela Vista and Alto Liro, which are located on the cliff (Figure 1).

Longitudinal surveys were implemented for two years (2005-

Figure 2. *Anopheles* larval habitats in Lobito, A: lower part of the town, A1: Caponte District, A2: San João District; B: upper part of the town, B1: Bela Vista District, B2: a water tank in Bela Vista District.

2006) in the houses of Sonamet workers who had previously had malaria infections confirmed by the company's medical department. In 2004, Sonamet began a comprehensive effort to reduce the high number of previously reported malaria cases among their staff and their families. They established a parasitological laboratory in the company's medical unit in order to ensure confirmatory diagnosis of all suspected cases prior to treatment. From June, 2004, the company also distributed LLINs to its staff and their families, together with malaria IEC, in an attempt to reduce the number of malaria infections. Consequently, a two-year longitudinal study was set up to evaluate entomological, parasitological, and immunological impacts resulting from the use of LLINs in this setting. The study was conducted from January, 2005 to December, 2006, in the Bela Vista District where clusters of malaria cases were recorded. A random sample of 21 families, including 155 people, of which 63 were children, were surveyed on a regular basis (every six weeks) throughout the study period. The 155 people slept in 59 sleeping units, to which 68 LLINs had been distributed. The scale of Sonamet LLIN distribution was restricted to families of its staff only. These families lived in an area inhabited by several thousand other people. The LLINs were provided with the aim of achieving individual protection rather than community protection. A control group consisting of 21 families that were neighboring, but not related to Sonamet employees and not processing LLINs, was selected and also monitored. These families received LLINs in the second year of the study (January, 2006).

Entomological evaluation was based on the monthly use of CDC LT inside houses with/without LLIN. In 2005, CDC LT monitoring was regularly implemented in six randomly selected houses among the 21 houses of the Sonamet employees with LLIN (called "Sonamet +") and in six nearby control houses (called "Sonamet –"). In 2005, 32 night catching sessions with six CDC LT (one per house) were conducted, achieving a total of 192 "night-traps." These included 16 sessions (96 night-traps) inside Sonamet + houses (LLIN since June, 2004) and 16 inside Sonamet – houses (without LLIN). In the same manner, in 2006, 34 night catching sessions were conducted using six CDC LT, achieving a total of 204 night-traps, in the same houses. These included 18 sessions with six CDC LT (108 night-traps) in Sonamet + houses, and 16 (96 night-traps) in Sonamet – houses (LLIN since January, 2006).

Morphological identification was based on the classical key of the Anophelinae of Africa south of the Sahara (Gillies and Coetzee 1987, Gillies and de Meillon 1968). Molecular identification of members of the Gambiae Complex was achieved using the RFLP-PCR assay (Fanello et al. 2002) with *HhaI* restriction enzyme which allows the differentiation of *An. coluzzii* and *An. gambiae*, as well as the other members of the complex. Infectivity rate was estimated by circumsporozoite (CSP) ELISA analysis (Burkot et

al. 1984, Wirtz et al. 1987). Each CSP ELISA 96-wells plate had a column of eight different negative controls that provided a median value used to calculate the cut-off value as three times the median value. An optical density was considered positive when its value was greater than this cut-off value. One well with the positive control was also added on each plate.

Statistical analysis

The χ^2 test was used for comparison of two proportions and the Exact Fisher test for the analysis of contingency tables, especially employed when sample sizes are small. All differences were considered significant at $p<0.05$.

RESULTS

Mosquito surveys

Considering main species only, among the 2,845 adult mosquitoes collected, a total of 155 *Anopheles* (5.4%) were captured along with 2,498 *Culex quinquefasciatus* (87.8%) and 192 *Aedes aegypti* (6.8%) (Table 1). The sample of *Anopheles* included four species, the dominant one was *An. melas* (34.8%), followed by two species of the Gambiae Complex, *An. coluzzii* (24.5%) and *An. gambiae* (22.6%), and *An. listeri* (18.1%). During the dry season, 122 *Anopheles* specimens were collected, including *An. melas* (44.3 %), *An. coluzzii* (29.5%), *An. gambiae* (4.9%), and *An. listeri* (21.3%); and among the 42 specimens of the Gambiae Complex, *An. coluzzii* was largely predominant (85.7%) compared to *An. gambiae* (14.3%). During the rainy season, 33 *Anopheles* specimens were collected, among which the majority was *An. gambiae* (87.9%), while *An. melas* was absent. In the upper part of Lobito, among the 52 *Anopheles* collected, 50% were *An. gambiae*, 17.3% *An. coluzzii*, 28.8% *An. melas*, and 3.9% *An. listeri*. In the lower part of the town, 103 *Anopheles* specimens were collected, including *An. melas* (37.9%), *An. coluzzii* (28.2%), *An. listeri* (25.2%), and *An. gambiae* (8.7%). The overall surveys of *An. gambiae* s.l. showed a nearly equal proportion of *An. coluzzii* (52%, n = 38) and *An. gambiae* (48%, n = 35). However, the presence of each species was seasonal and locational dependent. *Anopheles gambiae* predominated during the rainy season (82.9%) and in the upper area (74.3%), with its larvae found in domestic water storage tanks. In contrast, *An. coluzzii* was predominant during the dry season (94.7%) and in the lower part of Lobito (76.3%), where larvae were found in natural ponds and temporary pools. The same spatio-temporal variations occurred for both brackish water species, *An. melas* and *An. listeri*, which were collected in higher numbers during the dry season, 100% and 92.9%, and in the low part of Lobito, 72.2% and 92.9%, respectively (Table 1). In Caponte District, larvae of *An. coluzzii*, *An. gambiae,* and *An. melas* were found sympatrically.

Among the 155 *Anopheles* specimens collected, 119 were tested by CSP ELISA for the presence of circumsporozoites (malaria infectivity). None of the 28 specimens of the non-vector *An. listeri* were included in the analysis, nor small sample sizes such as those collected in March-April, 2004 and November-December, 2005 in the low part of Lobito (Table 1). Out of the 119 specimens of *An. melas* and *An. gambiae* s.l., only one *An. coluzzii* collected by IRF method in Sao Joao (low area) during the June-July, 2003 survey was found positive for *Plasmodium falciparum*. Overall, the infectivity rate of *An. coluzzii* was 3.9% (n=26) and 1.54% for the 65 specimens of *An. gambiae* s.l. analyzed.

Longitudinal surveys

A two-year longitudinal survey was implemented every month from January to December, 2005-2006, to evaluate the efficacy of LLIN. In 2005, the sample obtained by CDC light traps inside Sonamet + houses (LLIN since June, 2004) and in Sonamet – houses (no LLIN), was quite similar with respectively 39% and 37% of *An. coluzzii* and 61% and 63% of *An. gambiae* (Table 2). The presence of LLIN did not influence the ratio of *An. coluzzii* vs *An. gambiae*. In 2006, among the 154 *An. gambiae* s.l. collected by CDC light traps inside Sonamet + houses, the dominant species was *An. coluzzii* (79%), while in nearby Sonamet – houses (LLIN since January, 2006), the 122 *An. gambiae* s.l. collected showed an equal number of *An. coluzzii* (51%) and *An. gambiae* (49%) (Table 2). The results showed that in the same houses with traps implemented at the same time of the year, for two consecutive years, the ratio *An. coluzzii* vs *An. gambiae* significantly changed ($\chi^2 = 22.99$; $p< 0.05$), with a clear reduction of *An. gambiae* from 62% in 2005 to 35% in 2006. This change was apparently unaffected by LLIN ownership.

In 2005, in Sonamet + houses, three specimens were *P. falciparum* positive, two *An. coluzzii* (5.9%), and one *An. gambiae* (1.9%); while in Sonamet – houses, one *An. gambiae* (4.2%) was positive (Table 2). The infectivity rates were similar in the Sonamet + houses (3.4%; n = 88) and the Sonamet – houses (2.6%; n= 38) (exact Fisher test: 0.65). The infectivity rates were also similar in *An. coluzzii* (4.2%; n = 48) and *An. gambiae* (2.6%; n = 78) (exact Fisher test: 0.98). The total infectivity rate for 2005 was 3.2% (n = 126). In 2006, in houses Sonamet +, only one *An. gambiae* (4.4%) was found *P. falciparum* positive, while in Sonamet – houses, four specimens were positive, one *An. coluzzii* (1.6%) and three *An. gambiae* (5.1%) (Table 2). Overall, the infectivity rate in Sonamet + houses was 0.90% (n = 111), demonstrating an interesting reduction, although not significant, from the 3.4% reported in the first year (exact Fisher test: 0.23). The infectivity rate in Sonamet – houses (n= 120) was 3.3%, similar to the 2.6% of the previous year without LLIN (exact Fisher test: 0.65). The infectivity rates were similar in Sonamet + and Sonamet - houses (exact Fisher test: 0.21) and borderline but significantly different in *An. coluzzii* and *An. gambiae*, respectively 0.7% (n = 149) and 4.9% (n = 82) (exact Fisher test = 0.054). The overall infectivity rate in 2006 was 2.2% (n = 231), similar to the 3.2% of the previous year (exact Fisher test = 0.39). For both years, the infectivity rate of *An. gambiae* s.l. was 1.7% (n = 521).

Larval surveys

In March-April, 2004, in Caponte District (low part), larvae of *An. coluzzii*, *An. gambiae,* and *An. melas* were found sympatrically in three natural breeding sites (temporary pools) with a significant predominance of *An. coluzzii* (85%) over *An. gambiae* (11%). Among the 21 concrete water tanks covered with corrugated iron that were identified in Bela Vista (upper part) in 2005, 16 were positive for *Anopheles* larvae and all 30 specimens were to *An. gambiae* (Figure 2B). During January, 2007, larval surveys conducted in Liro (low part) reported nine positive breeding sites (natural water pools) and the adults from the collected larvae

Table 1. Adult mosquito collection data for the four sampling surveys conducted from 2003 to 2007 during the dry and rainy seasons, in the lower and upper parts of Lobito (total average of 24 collecting sites), in relation to the collecting methods.

Survey	Months Year	Period	Location	Method[1]	*Anopheles*, *Culex* and *Aedes* species[2]					
					An. melas	*An. coluzzii*	*An. gambiae*	*An. listeri*	*Cx. quinq.*[3]	*Ae. aegypti*
1	June-July 2003	dry	up	CDC LT	**7**	**2**	0	0	215	2
				HLC	**4**	**3**	0	1	438	0
				IRF	**4**	**4**	0	1	182	1
			low	CDC LT	0	0	0	0	13	0
				HLC	**1**	**2**	0	0	584	2
				IRF	**18**	**15**	0	2	557	62
2	March-April 2004	rainy	up	CDC LT	0	0	3	0	78	1
				HLC	0	0	0	0	0	0
			low	CDC	0	0	1	0	65	0
				HLC	0	0	0	0	21	116
3	Nov.-Dec. 2005	rainy	up	CDC LT	0	0	**23**	0	139	2
			low	HLC	0	2	2	2	30	0
4	Jan.-Feb. 2007	dry	low	CDC LT	**20**	**10**	**6**	22	176	6
Total					54 (34.8%)	38 (24.5%)	35 (22.6%)	28 (18.1%)	2,498	192
Total (%)						155 (5.4%)			2,498 (87.8%)	192 (6.8%)

[1]CDC LT, CDC light trap; HLC, Human landing collection; IRF, Indoor residual morning fauna.
[2]In bold, numbers of collected mosquitoes used in ELISA tests for infectivity rates.
[3]= *Culex quinquefasciatus*.

were identified as *An. listeri* (129 specimens), *An. melas* (47), *An. coluzzii* (27), and *An. gambiae* (19) associated with 413 *Culex* spp. In May, 2007, two positive breeding sites were noticed in natural ponds found in Liro where 17 *An. listeri* and 92 *Culex* spp. were collected.

DISCUSSION

A large number of studies on malaria (Cambournac et al. 1955, Ribeiro et al. 1964) and mosquitoes (Ribeiro 1966, 1969, Ribeiro and Ramos da Cunha 1975) were conducted in Angola from 1955 to 1975. After a gap of more than two decades due to civil war, studies commenced again on malaria case management and malaria morbidity (Rowe et al. 2009), entomological observations (Calzetta et al. 2008, Carrara et al. 2002, Choi and Townson 2012, Cuamba et al. 2006, Santolamazza et al. 2008), and vector control through indoor residual spraying (Somandjinga et al. 2009). In recent entomological surveys in Angola, *An. coluzzii* was reported as the most common species, present in dry and semi-arid areas of the provinces of Benguela, Luanda, and Namibe; while *An. gambiae* occurred in tropical humid environments along the coast (provinces of Bengo, Cabinda, and Zaire), and inland (provinces of Cuanza Norte, Malanje, Lunda Sul, and Huambo) (Calzetta et al. 2008, Carrara et al. 2002, Cuamba et al. 2006, Santolamazza et al. 2008), although not previously reported from Lobito. Our surveys showed the presence of sympatric populations of *An. coluzzii* and *An. gambiae* in the lower (San Joao) and upper districts (Alto Liro) (Toto et al. 2011) of Lobito. Overall, *An. coluzzii* was dominant during the dry season and in the lower part of the town (Restinga, Lobito Velho, Sonamet Yard, Caponte, Liro, and Sao Joao), while *An. gambiae* was mainly present during the rainy season and in the upper part of the town (Alto Liro and Bela Vista). The same seasonal (dry) and spatial (low) pattern for *An. coluzzii* was also observed for *An. melas* and *An. listeri*, two brackish water species. In contrast, the upper part of Lobito is dry and inhabitants had to build their own water storage cisterns to supply their homes. These

Table 2. Data collected during the two-year longitudinal surveys (2005 and 2006) in the Bela Vista area (upper Lobito).

		Anopheles gambiae s.l. (Frequency %)			*P. falciparum* positive (Infectivity rate %)		
	Total mosquitoes	Total/Total analyzed	*An. coluzzii*	*An. gambiae*	*An. coluzzii*	*An. gambiae*	Total
			2005*				
Sonamet +	1038	96/88 (9%)	34 (39%)	54 (61%)	2 (5.9%)	1 (1.9%)	3 (3.4%)
Sonamet -	1166	149/38 (13%)	14 (37%)	24 (63%)	0	1 (4.2%)	1 (2.6%)
Total	2204	245/126 (11%)	48 (38%)	78 (62%)	2 (4.2%)	2 (2.6%)	4 (3.2%)
			2006*				
Sonamet +	1848	154/111 (8%)	88 (79%)	23 (21%)	0	1 (4.4%)	1 (0.9%)
Sonamet -	1433	122/120 (9%)	61 (51%)	59 (49%)	1 (1.6%)	3 (5.1%)	4 (3.3%)
Total	3281	276/231 (8%)	149 (65%)	82 (35%)	1 (0.7%)	4 (4.9%)	5 (2.2%)
TOTAL	5485	521/356 (10%)	196 (55%)	160 (45%)	3 (1.5%)	6 (3.8%)	9 (2.5%)

2005 Sonamet +: six houses with LLIN since June, 2004; Sonamet -: six houses without LLIN.
2006 Sonamet +: six houses with LLIN since June, 2004; Sonamet -: six houses with LLIN since January, 2006.

cisterns constitute remarkable larval habitats especially suitable for *An. gambiae*, although some larvae of *An. coluzzii* were also sympatrically collected in these sites. The presence of larvae of *An. gambiae* in man-made cisterns provides a clear rational for the use of larval control in this part of Lobito.

The two-year longitudinal survey implemented during 2005-2006 in the Bela Vista area, where clusters of malaria cases occurred among Sonamet employees and their families, allowed a comprehensive biological analysis including variation in plasmodic indices and the levels of salivary protein antibodies in people with and without LLIN (Drame et al. 2010b). Entomological trapping, primarily with CDC light traps, collected 521 specimens of *An. gambiae* s.l. during the two-year surveys, of these 47% were captured in 2005 and 53% in 2006. The overall proportions of *An. coluzzii* and *An. gambiae* were quite similar, respectively 55% and 45%. However, a change in proportion was noticed, with *An. gambiae* predominant the first year (62% in 2005) switching to *An. coluzzii* the second year (65% in 2006); and this observation was independent of LLIN ownership. Environmental anthropic modification in the Bela Vista area may have caused the observed decrease of *An. gambiae* and the increase of *An. coluzzii*. The installation of tap water systems during our surveys in some districts, such as Bela Vista, resulted in domestic water cisterns falling into disrepair, being dried out, and serving as rubbish dumps. The reduction in number of man-made larval habitats, particularly suitable to *An. gambiae*, was noted during the regular surveys and reported by GPS localization (Almeida et al. unpublished data) to guide National Malaria Control Programme larval control activities using *Bacillus thuringiensis israelensis* (Bti). It is possible that this modification in environmental conditions and change in human behavior contributed significantly to the subsequent changes observed in the proportions of *An. coluzzii* and *An. gambiae*.

These entomological surveys elucidated the epidemiology of malaria in Bela Vista at a micro scale, a dry area on the cliff of Lobito that previously seemed to be an unsuitable habitat for *Anopheles* mosquitoes for two reasons: (1) it is an urban area when *Anopheles* mosquitoes are mostly occurring in rural areas (or semi-urban); (2) the upper part of Lobito is extremely dry with conditions that are quite harsh for these mosquitoes. It is because of this dryness that inhabitants of the upper part of the town built cement domestic tanks to make up for the lack of water. These human-made water storage systems built close to houses provided a good breeding habitat for vector mosquitoes, as shown in this study, resulting in year-round transmission of malaria to nearby households.

The infectivity rates found during our longitudinal surveys varied from 3.41% in 2005 to 0.90% in 2006. Within *An. coluzzii*, the infectivity rates ranged between 0.67% and 5.88%, while for *An. gambiae* the rates varied from 1.85% to 5.09%. These *Plasmodium* infectivity rates in Lobito are comparable to those previously reported (Calzetta et al. 2008) within the *An. gambiae* s.l. samples from three provinces of Angola. When comparing the overall *Pf*-CSP positivity of the *An. gambiae* s.l. specimens

found during both the mosquito and the longitudinal surveys, our respective values of 1.54% (n = 65) and 2.53% (n = 521) were not significantly different from those previously reported such as 0.4% (Carrara et al. 2002), 0.59% (Calzetta et al. 2008), and 1.9% (Cuamba et al. 2006).

The relatively low number of LLIN distributed in Bela Vista was insufficient to create a mass effect. This was, however, not the intent of this targeted LLIN distribution program, which aimed simply to provide individual protection of Sonamet employees and their families. The National Malaria Control Programme plans to ensure universal LLIN coverage for the population. This study also showed that, according to CDC light trap samples, LLIN did not reduce either the number or the infectivity rate of *An. gambiae* s.l. caught inside houses in Lobito town, while immunological tests showed in another study done in the rural area of Balombo, Angola, that LLIN did reduce significantly the man-*Anopheles* contact (Brosseau et al. 2012). This information confirms the usefulness of this new immunological biomarker for improving the evaluation of LLIN and other vector control methods, and comparing their efficiency (Brosseau et al. 2012, Drame et al. 2010b).

It is notable that the samples obtained by CDC light traps during the two-year longitudinal study in Bela Vista confirmed the transmission of *P. falciparum* during the dry season and in a dry area that was apparently not favorable, at the macro-ecologic level, for the development of *Anopheles* species. The presence of *An. coluzzii* and *An. gambiae* justifies targeted vector control operations to reduce the burden of malaria in Lobito, and possibly other similar ecological settings within Angola.

During these entomological investigations, specimens of *Culex quinquefasciatus* and *Cx. neavei*, vectors of West Nile virus were also collected, as well as *Aedes aegypti*, the main vector of yellow fever, dengue, and other arboviruses (i.e., Chikungunya). The presence of these vectors should be the subject of further investigation, because it is possible that many fevers are misreported as "clinical malaria" when in fact the clinical symptoms may be caused by arboviruses. Therefore, in order to plan and achieve effective malaria control, good quality diagnostic services producing accurate disease surveillance data, combined with a solid entomological evidence base, are vital to ensure a measured and targeted package of effective control interventions tailored to the local epidemiological context.

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