Characterization of Potential Larval Habitats and Spatial Distribution of Anopheles Species Larvae During the Dry Season in Mutare City, Zimbabwe: a Cross-sectional Study

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Abstract

Background: Malaria is traditionally known to be concentrated in rural areas but the disease is increasingly becoming a major public health problem for urban settlements in Africa. National malaria reports show that Mutare City had the greatest endemic malaria burden among all urban settlements in Zimbabwe. To prevent malaria outbreaks, it is essential to monitor malaria vectors in populous urban centers to establish the foci of the vectors while they are still small. This study sought to identify, describe, quantify, geocode mosquito potential larval habitats and their spatial distribution within the city.

Methods: Mutare City was divided into four regions and the study was conducted from June to November 2019. Larval sampling of 223 potential habitats was done and the collected larvae were reared until they became adult mosquitoes before being morphologically identified using the keys developed by Gillies and Coetzee. Data were entered and analyzed using Epi Info version 7.2.1.0 statistical package.

Results: Anopheline speciation showed that the city was infested with *Anopheles funestus* (4.9%), *An. arabiensis* (0.3%), *An. pretoriensis* (91.3%) *An. coustani* (0.5%), *An. rufipes* (2.8%), and *An. maculipalpis* (0.2%). Overall, *An. funestus* group was the predominant with *An. arabiensis* complex less common. The species composition of *An. funestus* group varied significantly among the sampling habitats. Results showed most mosquito breeding habitats were due to human activities such as agriculture, earth mining, and leaking piped water valves. The digging of shallow wells for domestic use in the high-density areas also contributed to the increase in mosquito breeding habitats.

Conclusion: The most significant malaria vectors in the city were *An. funestus* ss and *An. arabiensis*. The proliferation of larval habitat hotspots in Mutare City was being fueled by human activities related to earning a living and housing construction. Effective interventions for enhanced larval source management could use a multi-sectoral approach involving all the urban stakeholders. Considering the rapid expansion of the city, more investment is needed to target the most productive habitats by fixing leaking water pipes and frequent application of larvicides with greater residual activity to treat permanent habitats particularly before the rainy season.

Background

In 2017, there were an estimated 219 global million cases of malaria and 435 000 of these cases succumbed to the disease during the same year [1]. The same report revealed that African countries carried a disproportionate burden of the disease reporting an increase in malaria cases with 10 highest-burden African states recording about 3.5 million more malaria cases compared to the previous year. Two years later in 2019, the global number of malaria cases jumped to 229 million with the World Health Organization (WHO) African Region accounting for about 94% of the cases (215 million) [2]. Despite the diverse efforts and massive investments to prevent and control malaria, the disease remains a major public health challenge causing immense mortality and morbidity which are major barriers to socio-economic development.

The transmission of malaria is naturally heterogeneous [3] and urbanization is one of the factors posing new malaria disease control challenges [4]. Traditionally, malaria was believed to be a rural disease since the *Anopheles* mosquitoes had abundant breeding habitats in rural areas [5]. Limited economic opportunities in the rural areas have resulted in rapid, unplanned, and uncontrolled rural-to-urban migration in most African cities and this subsequently led to the deterioration of socioeconomic and environmental conditions in the cities [6]. Consequently, the uncontrolled sprawling urban slum expansion produced new larval habitats where vectors flourish and contribute to malaria transmission in formerly low malaria burden environments. It is important to determine the presence as well as density of the various malaria vector species because the findings can potentially feed into the success of efforts to eliminate the disease by understanding the dynamics, patterns, and abundance of the vector habitats. While malaria is predominantly a low and middle-income country problem, significant studies have been conducted in the developed countries and not in the affected continents like Africa [7, 8]. Malaria causes a substantial economic burden and investing in fighting the disease can improve economic gains.

Despite predictions that urbanization will lead to reduced malaria transmission due to improved housing and better healthcare, some African cities are recording more cases than the surrounding rural areas [9]. Many African countries are unprepared to deal with the rapid spread of malaria in their towns and cities due to the increased mobility between rural and urban areas, and poorly resourced healthcare systems. Unpaved roads and poor quality housing in low socioeconomic environments particularly in the peripheries of the cities create a conducive environment for the malaria vectors to thrive [10–13]. The adaptation of malaria vectors to urban ecosystems warrants close attention in sub-Saharan Africa [4].

Although the sub-Saharan Africa Region is resident to more than 128 indigenous *Anopheles* (*An.*) species [14], only six are responsible for 95% of all malaria transmission on the continent [15]. The identified species are *An. gambiae, An. arabiensis, An. coluzzi, An. funestus, An. moucheti* and *An. nili* [15, 16]. A malaria vector survey and vector surveillance survey done in Limpopo Province of South Africa (bordering Zimbabwe) reported that *An. arabiensis* was a prime malaria vector in the area [17]. Sande et al. (2015) identified that *An. funetus sensu stricto* and *An. arabiensis* were the most important vectors of malaria transmission in Mutare and Mutasa Districts of Zimbabwe [18] while Zengenene et al., (2020) identified the *An. funestus* as the only malaria vector in their study conducted in Chiredzi District, Zimbabwe [19]. A similar ecological study in Kenya urban reported that larval sites were man-made and the largest proportion of habitats were observed in unplanned, and poorly drained areas [20]. Studies elsewhere also indicated that market-gardening wells were important larval habitats for *An. arabiensis* [5, 21].

The risk of malaria infection in Zimbabwe is driven mainly by climatic covariates that vary widely according to geographic region, season, and year [21]. Malaria epidemiology varies across the country ranging from year-round transmission in lowland areas to epidemic-prone highland areas. Manicaland Province is one of the three provinces with the highest malaria prevalence in Zimbabwe. According to the Zimbabwe District Health Information System 2 data of 2016 (unpublished), approximately 82% of all malaria cases in 2016 predominantly originated from three eastern provinces of Zimbabwe namely, Manicaland, Mashonaland East, and Mashonaland Central, with 39% of all cases and 31% of all deaths coming from Manicaland Province. This trend has been consistent in Zimbabwe since 2013. The Zimbabwe Malaria Stratification Map 2015 indicated the risk of malaria as lowest in the north-south central region and increased towards the country's borders with Mozambique and Zambia [22].

According to data from the Mutare City Health Department (unpublished), trends of malaria disease in the city showed that cases in the city's suburbs remained above zero indicating a low but insidious local transmission since the outbreak of the disease in 2017. Sporadic outbreaks were observed at the council clinics with cases increasing above the threshold level at all clinics during week 1 to week 8 of 2017. This prompted the City Health Department to conduct identification of vector breeding sites and indoor residual spraying (IRS) in the most affected suburbs resulting in fewer cases during the 2018 malaria season when only Dangamvura Clinic reported cases above the threshold value during week 47. Intermittent above threshold levels of malaria cases sprouted from week 20 through to week 50 of 2019. The spasmodic occurrence of malaria cases in the city signaled the presence of unidentified vector breeding sites which warranted attention.

To mount effective and organized vector control measures, it was imperative to investigate and map these vector breeding sites. It is essential to monitor malaria vectors in populous urban centers to establish the foci of the vectors while they are still small to prevent recurring malaria outbreaks [23]. Studies have shown that it is significantly cost-effective to use larval source management (LSM) strategies than other malaria control measures in Africa. Although interventions against malaria are complementary, three LSM programs showed that the cost of protecting one person from malaria each year in Africa ranged from US$0.94 to UD$2.50 [24] when compared to indoor residual spraying (US$0.88 to US$4.94) [25] and long-lasting insecticide-treated nets (US$1.48 to US$2.640 [26]. This study sought to identify, describe, quantify, geocode mosquito potential larval habitats and their spatial distribution within the city's jurisdiction.

Methods

Study Area

The study was carried out in Mutare City located at a latitude of 18^{o}5'0" and longitude 32^{o}40'0" (estimated population: 262 124) (Fig. 1). Mutare is Zimbabwe's fourth-largest city located 265 kilometers to the east of Harare and lies north of the Vumba Mountain ranges that extend into Mozambique. The city covers an area of 191km^2 and has a population density of 981.3/km^2. It has a temperate climate with an average annual temperature of 19°C and moderate altitude (1110 m). The rains in the area are mostly received in the months of mid-November to April each year and the city receives more than 1000 mm of rainfall annually. Mutare City has a high groundwater table and is dotted with marshes, swamps, and ponds, combined with disturbances resulting from human activities, provides excellent breeding habitats for mosquitoes. The local ecological features of the area are predominantly savanna grassland and woodland ecosystem and the selection of breeding sites was mainly based on the ecological condition known to be potential breeding habitats for the vector. The study leveraged on the existence of laboratory and insectary facilities at Africa University and an already assembled field team of Scouters as well as community and logistical support from the Mutare City Council.

The area along the Zimbabwe-Mozambique border continues to bear the biggest burden of malaria [27]. This porous frontier lacks defined demarcations between the two countries and citizens from either side crosses the borders easily without formal documentation. This causes an uncontrolled entry and exit of traders between the two countries making it difficult to control the disease. Zimbabweans frequently visit Mozambique to buy goods for resale while Mozambicans cross into Zimbabwe in search of casual opportunities in commercial farms [28]. A study by Chiruvu et al., (2017) revealed that the proportion of patients from Mozambique that were treated for malaria in Zimbabwean health facilities increased from 2013–2015 [28]

Study Period

The collection and rearing of mosquito larvae were done during the dry season from 1 June to 5 November 2019 that is from winter up to the end of the dry season.

Entomological Sampling

The researchers stratified Mutare City into Northern, Southern, Eastern, and Western Regions. The four regions had different population densities with the Western and Southern regions experiencing dynamic changes in demography due to unplanned and uncontrolled urban expansion. Trained mosquito breeding site scouting experts employed by the Mutare City were employed to identify the potential vector breeding habitats. The Scouters also resided in their respective suburbs and had good knowledge of the area. Each of the four regions was visually inspected for the presence of water bodies and what influenced their creation. At least three Scouters did a thorough search of outdoor potential breeding sites for each residential suburb. These potential breeding habitats for the different regions were further stratified into man-made artificial (e.g. construction, earth mining, leaking pipe sites, storage containers, ponds) or natural origin (e.g. streams, dams, swamps) aquatic habitats. The breeding habitats for each stratified region were noted down as the sampling frame for that area and each breeding habitat was allocated a computer-generated number. All mosquito breeding habitats were identified with GPS coordinates of the site before being recorded on the survey form. A potential vector breeding habitat was defined as an accessible stagnant open water source conducive to *Anopheline* larval breeding.

Table 1
Mutare City stratification by region.

Stratification (Regions)	Residential suburbs
Northern Suburbs	Murambi, Fairbridge Park, Tiger's Kloof, Avenues, Yeovil, Westlea, Florida, Central Business District
Southern Suburbs	Sakubva; Dangamvura, Weirmouth; Fern Valley, Darlington, Nyakamete Industrial Area, Muneni, Gimboki
Eastern Suburbs	Bordervale, Morningside, Palmerston, Greenside
Western Suburbs	Utopia, Chikanga, Hob-House, Natview Park; Zimta Park

Sampling size determination of the potential breeding habitats took environmental heterogeneity into consideration. The target sample size was determined using Fisher's formula. For equal allocation of site, a design effect of 3 [30] was utilized in calculating the minimum sample size. This study also used the most conservative estimate of p (0.5) [31], an alpha level of 95% (1.96), a maximum tolerable error of 10%, and a precision of 0.05 in sample size calculation. Thus, $n = 3(1.96)^2 \times 0.5(1- 0.5)/ 0.10^2$ yielded an average sample size of 288 breeding sites. This study used an acceptable standard deviation of 23% and the ultimate sampling range was therefore 66 ± 288.

Habitat Characterization

The potential larval habitats were extensively searched for mosquito larval stages, specifically for immature aquatic stages. The habitats were characterized by coverage of canopy, surface debris, emergent plants, water depth, and habitat types. Sampled habitats without larvae present were recorded as "negative habitats", those with larvae were recorded as "positive habitats". Habitats with perimeters larger than 10 m were sampled at multiple stations between 5 m apart. For such habitats, only one geolocation of the sampling stations was recorded to represent the whole habitat. Environmental parameters included abiotic and biotic categories were further defined by sub-categories provided by a table. Habitats were classified into man-made and natural sites. The sampling and characterization of the potential larval habitats were done twice for bodies around residential suburbs if the first survey showed no results and the station was a well-known hotspot of invasive species from previous collections.

Abiotic characteristics included the water depth -shallow (< 1 meter deep), deep (≥ 1 meter deep); Sunlight- semi-shade, shade, deep shade; debris present or absence, and type of breeding site- river-bed, stream, dam, swamp-like seepage, hoof print, rain pool. Biotic parameters were classified by the canopy (vegetation above habitats), vegetation (living within the aquatic habitat), and these vegetation classifications were-none, floating vegetation, and emergent (emergent plants, floating, none). To maintain consistency, all visual classifications were done by one person who was also the principal investigator.

Collection and rearing of mosquitoes larva

A standard larvae collection form was used while geocoding and site description was done for each selected breeding site. The standard dipping method [32] was used to collect mosquito larvae utilizing 250 ml dippers times 10 dips taken at each habitat station. The mosquito larvae were placed in plastic jugs soon after larval density assessment and morphological determination of species. *Anopheline* larvae were separated from *Culicines.* The jugs were transported to the Africa University insectaries where they were reared according to the WHO (2003) guidelines [33]. The larvae were pooled according to region and habitat where they were obtained from. The mosquito larvae were screened morphologically and counted using taxonomy keys of Gillies and De Meillon [34]. Collected larvae were raised to adults and live *Anopheles* mosquitoes were killed by freezing before identification to species level by using morphologic keys developed by Gillies and Coetzee [35]. For post morphological identification, the mosquito samples were preserved individually in vials with silica gel for molecular differentiation of species.

Data analysis

Obtained data were entered and analyzed using Epi Info version 7.2.1.0 statistical package.

Results

Anopheline larvae were sampled from 223 potential larval habitats from the Mutare City. The distribution of the sampled potential breeding habitats is shown in Table 2.

Table 2
Potential larval habitats distribution in Mutare Urban showing the potential breeding habitats sampled per region and percentage relative to the total number of samples.

Region	Number of sampled habitats, n (%)	Residential suburb	Potential breeding *Anopheline* habitat sampled/suburb
Eastern	55 (24.7)	Bordervale	9
		Morningside	15
		Palmerston	2
		Greenside	29
Western	48 (21.5)	Chikanga	38
		Hobhouse	7
		Zimta Park	2
Northern	62 (27.8)	Murambi	20
		Fairbridge Park	12
		Avenues	7
		Yeovil	9
		Westlea	3
		Florida	11
Southern	58 (26)	Mutare CBD	10
		Sakubva	12
		Dangamvura	11
		Weirmouth	2
		Fern Valley	4
		Darlington	5
		Gimboki	4
		Nyamakate	12

Mosquito Identification and Distribution

Culicines were the most abundant type of mosquito breeding among most of the potential breeding habitats in the city. Table 3 shows the species identification and distribution according to residential suburbs. Adult mosquitoes were identified morphologically to family level and further analyzed by molecular means. Figure 2 shows the sampled potential larval habitats and the sites where Anopheline larvae were identified. *An. funestus s.l.* (malaria-causing species) and *An. pretoriensis* (a non-malaria vector species) were the most prevalent vector species found in the surveyed residential suburbs. Other species collected were *An. pretoriensis*, *An. coustani*, *An. rufipes*, *An. maculipalpis* and *An. arabiensis.* Sympatry in Culicine and anopheline larvae were found in 35% (77/223) of the habitats in Mutare City suggesting that the vector larvae from the *Anophelinae* and *Culicinae* sub-families coexist in more than a third of the habitats.

Table 3
Summary of species identification and distribution.

Suburb	*An. funestus s.l.*	*An. arabiensis*	*An. pretorien-sis*	*An. coustani*	*An. rufipes*	*An. maculip-alplis*	*Culicines*
Bordervale,	X				X		X
Morningside			X				X
Palmerston							X
Greenside	X	X	X	X		X	X
Hobhouse	X						X
Chikanga	X		X	X	X	X	X
Zimta park							X
Murambi			X				X
Fairbridge Park	X		X	X	X		X
Avenues			X				X
Yeovil			X				X
Westlea	X		X				X
Florida	X				X		X
Sakubva			X			X	X
Dangamvura			X	X		X	X
Weirmouth	X		X		X		X
Fern valley	X		X		X		X
Darlington			X				X
Nyakamete			X				X
Gimboki		X	X			X	X

There were many small man-made shallow wells in all high-density areas because tapped water supply was erratic. Gimboki had the highest number of these wells because the suburb did not receive any piped water from the city council. Activities (such as brick molding, quarrying, and gardening) known to cause artificial man-made open ponds were rife in most of the high-density suburbs particularly in the Western and Southern Regions. The high number of Culex species indicate an increased number of nuisance mosquitoes.

Anopheles Vector Species Habitat Classifications.

Table 4 shows the densities of Anopheline and Culicine mosquito larvae in the four identified regions of Mutare City. Anopheles vector species aquatic stages were found in a wide range of habitat classifications: namely swamps, seepage pools, burst municipal water pipes, river bed habitats, drains, ponds, and dams as shown in Table 5. The most commonly occupied habitats were swamps, burst municipal water pipes, and seepage pools.

Table 4
Mutare City larval density (mosquito larvae per 10 dips of 250ml dippers) per region.

Region	Anopheles	Density	Culicines	Density	Total	Region Density
Eastern region	114/550	0.21	538/550	1.0	652/550	1.2
Western region	934/470	2.0	1143/470	2.4	2077,470	4.7
Northern region	256/620	0.41	424/620	0.7	680/620	1.1
Southern region	416/580	0.72	750/580	1.3	1166/580	2.0
Average larval density		**0.84**		**1.35**		**2.25**

Table 5
Summary of larval habitats in the four regions of Mutare City with *Anopheles* larvae.

Habitat Classification	Type of habitat	Habitats	Positive	Percentage of positives
Man-made	Seepage pools	28	20	71.4
	Burst municipal water pipes/valves	114	22	15.3
	Construction Trenches/drains	4	3	75
Natural	Swamps	51	38	74.5
	Rivers/streams	17	10	58.8
	Dams	9	4	44.4
Total		**223**	**97**	**43.5**

Table 6
Residential suburbs with *Anopheles* larvae identified and their specific habitats.

Suburb	*An. funestus s.l.*	*An. arabiensis*	Habitats
Bordervale	X		LV
Greenside	X	X	SLS
Hob House	X		SLS
Chikanga	X		SLS, DM, RB
Fairbridge Park	X		LV, SLS
Westlea	X		LP
Florida	X		SLS, LP
Dangamvura	X		SLS, ST
Weirmouth	X		ST
Fern valley	X		RB, SLS
Gimboki		X	SLS, ST

Leakages of valves (LV), leaking/burst pipes (LP,) streams (ST), rivers beds (RB), dams (DM) seepage like swamp (SLS).

Discussion

The present study sought to identify, describe, quantify, geocode mosquito potential larval habitats and their spatial distribution within Mutare City to help in crafting interventions to reduce malaria transmission. *An. funestus* was the most prevalent of the *Anopheline* species. The erratic supply of water in the high-density suburbs had caused residents to seek alternative water sources for domestic use and gardening. These new water sources were usually in the form of open shallow wells. The Southern and Western Regions had the highest *Anopheline* larval densities. Man-made potential larval habitats were seepage pools and construction site trenches while swamps and streams dominated the natural habitats.

The *An. funestus* was the primary malaria vector in Mutare City as the results showed that An. funestus larvae colonized a large number of the water sources. This species which is known to have an indoor resting and biting characteristic and resistance to pyrethroids and carbamates were also identified in Mutasa District [18, 36] which is adjacent to Mutare City. Similarly, other studies in Zimbabwe [18, 37] and also noted that when the two malaria-causing vectors were identified, *An. funestus* was relatively more abundant than *An. gambiae s.l.* These two vectors are among the world's most efficient malaria vectors and also breed in sympatry.

Poor access to piped water supply in the Western and Southern Regions (high-density residential areas) when compared to the other regions resulted in high larval densities of the malaria-causing vectors. Unrepaired broken water pipes and shallow wells dug to access ground-water provided breeding habitats for the mosquito larvae. This suggests that larval habitat abundance is related to the socio-economic status of the area as noted in a similar study [20]. Thus, interventions to combat malaria should be concentrated in these areas of the city.

The informal nature of the economy in Mutare City played a pivotal role in the abundance and distribution of vector habitats in the study area as evidenced by the majority of the potential habitats identified being man-made. Stagnant residual water sources containing mosquito larvae were mostly seepage pools, housing construction trenches, earth mining, and gardening. This finding suggests that malaria transmission in Mutare City was from manipulation of the natural ecosystem by human modification as observed in other studies [18, 20]. Common ground, man-made drains, and wells tend to keep water for considerably longer times than natural habitats thereby providing the ideal conditions for Anopheline and Culicine larvae to breed [38].

Human settlements in the high-density suburbs were built on land that was not serviced that is, poorly drained, and lacking plumbing. This resulted in increased numbers of stagnant water pools which are convenient breeding habitats for mosquitoes. Thus, proper sanitation service, infrastructure, and housing development are key factors in intervening towards reducing malaria incidence in the study area. Installation of consistent water delivery and drainage systems can reduce the Anopheline habitats by eliminating the standing water available for larval proliferation. A systematic review by Keiser and colleagues reported that incorporating drainage of aquatic habitats into city development plans can potentially eliminate mosquito larval habitats in the long term [39]. Previous studies reported that garden wells were common Anopheline larval habitats [5, 40]. Although gardening was a vital practice to promote food security in the residential area, the farmers need to be monitored and educated on practices that minimize vector breeding.

The natural habitats of mosquitoes were mostly swamps and streams. Seepage like swamps were more likely to contain Anopheline larvae in Mutare City. This finding was consistent with what another study noted in Luanda, Angola [38].

Study Limitations

In Africa, mosquito densities fluctuate with seasons with a high prevalence of the vector being recorded during the rainy season [41]. This study was implemented from June to November 2019 and it is suspected that the rain season which is known for increased potential habitats was not covered therefore the researchers recommend a repeat in the summer season and subsequent result comparison. Due to the increased number of potential larval habitats in the study area, very small breeding sites may have been largely excluded from selection and this could potentially omit factors important for vector control interventions.

Conclusion

The most significant malaria vectors in the city were *An. funestus ss* and *An. arabiensis*. The proliferation of larval habitat hotspots in Mutare City was being fueled by human activities that are aligned towards earning a living and housing construction. Effective interventions for enhanced LSM could use a multi-sectoral approach involving all the urban stakeholders. Consistent malaria disease surveillance and controlled land use combined with environmental management can potentially lead to LSM which involves the management of larval aquatic habitats to curb the mosquitoes from breeding to maturity. Considering the rapid expansion of the city, more investment is needed to target the most productive habitats by fixing the leaking water pipes and providing adequate water to the population, and frequent application of larvicides with greater residual activity. The treatment of permanent habitats should be done in winter and shortly before the rainy season before the number of larval habitats multiplies rapidly.

List Of Abbreviations

IRS Indoor residual spraying

LSM Larval source management

WHO World Health Organization

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AM: lead author of the manuscript, **TP:** Coordinated study activities and reviewed the manuscript, **NM**: laboratory analysis and reviewed manuscript, **ML**: provided technical expertise and reviewed the manuscript: **MM**: supervised study activities and reviewed the manuscript, **SM:** study design, supervised the study. PTM wrote the draft manuscript. All authors read and approved the final manuscript.

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Figures

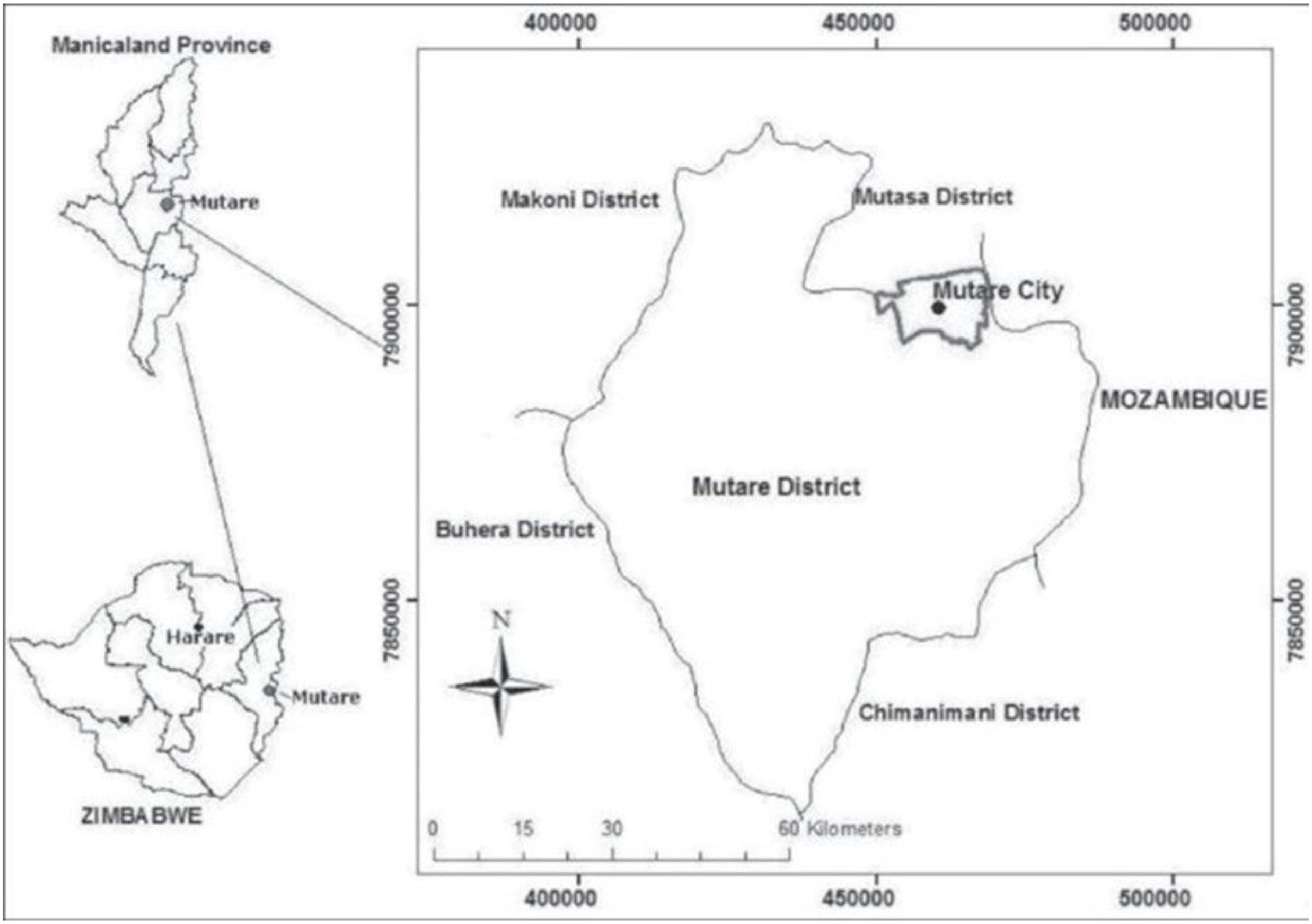

Figure 1

Location of Mutare City within Mutare District of Manicaland Province, Zimbabwe [29] Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

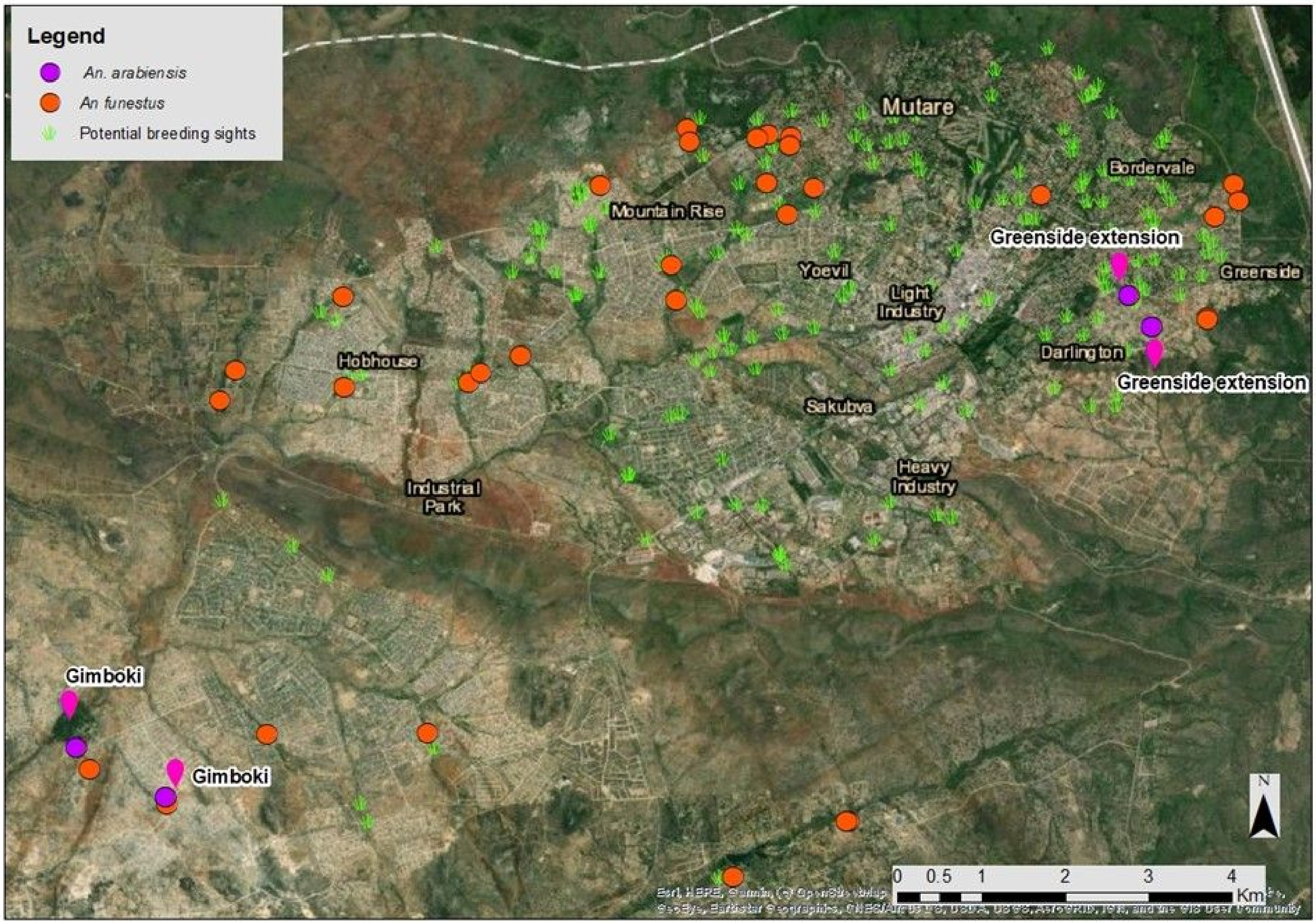

Figure 2

The sampled potential larval habitats distribution and the sites where An. arabiensis and An. funestus were identified in Mutare City. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 3

The different types of mosquito breeding habitats that were identified in Mutare City. Aquatic stages of mosquito were sampled in an incomplete house foundation (a), abandoned house foundation (b) around houses built in swampy areas (c), uncovered shallow wells (d) leaking sewer valves e, leaking fresh water pipes and (f), standing streams.