

Habitat characterization and insecticide susceptibility profiles of *Aedes aegypti* mosquitoes in Ifakara area, south-eastern Tanzania

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Abstract

Background: *Aedes*-borne diseases such as dengue and chikungunya constitute constant threats globally. In Tanzania, these diseases are transmitted by *Aedes aegypti*, which is widely distributed in urban areas, but whose ecology remains poorly-understood in small towns and rural settings.

Methods: We surveyed aquatic habitats of *Ae. aegypti* mosquitoes in and around Ifakara, a fast-growing town in south-eastern Tanzania. The area was divided into 200m × 200m search grids and habitats containing immature *Aedes* were characterized. Field-collected *Aedes* were tested for susceptibility to common public health insecticides (deltamethrin, permethrin, bendiocarb and pyrimiphos methyl) in dry and rainy seasons.

Results: Of 1,515 and 1,933 aquatic habitats examined in dry and rainy seasons respectively, 18.87% and 14.64% contained *Aedes* immatures (container index (CI): 286-283). In the 2,315 and 2,832 houses visited in dry and rainy seasons, 4.9% and 6.6% had at least one *Aedes*-positive habitat (house index (HI): 114-186). The main habitat types included: a) used vehicle tires and discarded containers, b) flower pots and clay pots, and c) holes made by residents on trunks of coconut trees to support climbing harvesters. Used tires had highest overall abundance of *Aedes* immatures, while coconut tree holes had highest densities per habitat. *Ae. aegypti* adults were susceptible to all tested insecticides in both seasons, except bendiocarb, against which resistance was observed in rainy season.

Conclusion: This is the first study on ecology and insecticide susceptibility of *Aedes* in Ifakara area, and will provide a basis for future studies on its pathogen transmission activities and its control. The high infestation levels observed indicate significant risk of *Aedes*-borne diseases, requiring immediate action to prevent potential outbreaks in the area. While used tires, discarded containers and flower pots are key habitats for *Aedes* here, this study also identified coconut harvesting as an important risk factor, and the associated tree-holes as potential targets for *Aedes* control. Since *Ae. aegypti* mosquitoes in the area are still susceptible to most insecticides, effective control could combine environmental management, preferably involving communities, habitat removal and insecticide spraying.

Background

In recent decades, significant attention has been put on controlling mosquitoes that transmit malaria, leading to significant progress since 2000 [1,2]. However, other mosquito-borne diseases, such as dengue, yellow fever, chikungunya and zika, which are transmitted by *Aedes* mosquitoes remain largely neglected. Golding *et al.*, 2015 showed that more than 90% of persons at risk of vector-borne diseases are affected by at least two such diseases, malaria and dengue fever being the commonest [3]. The WHO Global Vector Control Response (GVCR) initiative therefore recommended integrated approaches to address multiple vectors and vector-borne diseases [4]. Unfortunately, unlike malaria, for which effective prevention and treatment options are widely available, the *Aedes*-borne diseases still rely mostly on personal protection measures [5], even though vaccine trials are increasingly advanced as well [6].

In Tanzania, concerns about *Aedes*-borne diseases have become increasingly prominent in recent years, due to multiple outbreaks, detection of the viruses in humans, and the wide distribution of the *Aedes* mosquitoes [7–10]. Dengue cases have been reported in multiple regions in the country, including Dar es Salaam city, the

islands of Zanzibar and Pemba, Mbeya and Iringa areas in the southern Tanzania, and Kilimanjaro in the north [11–13]. The most recent outbreak occurred in May 2019, when 1,012 new cases were confirmed over just two weeks [14]. By September 2019, 6912 cases had been reported, including 13 deaths [14].

Most outbreaks of *Aedes*-borne diseases have been observed in urban areas, where densities of both the vector and humans are high [11]. However, human mobility has also led to introduction of the viruses in rural areas and small towns [7]. Unfortunately efforts against these diseases are hampered by lack of proper medication or diagnostics [15–17]. Effective vector surveillance and control to prevent potentially-infectious mosquito bites therefore remain core components of programs targeting such diseases [5].

Current understanding of *Aedes* mosquitoes is largely based on studies in urban areas where the vector is most widespread [18]. *Ae. aegypti*, the most important of the *Aedes* species, is considered highly anthropophilic, and is a frequent breeder in artificial containers [19], common in urban settings [8]. Improper disposal of waste containers provides perfect breeding environment for *Ae. aegypti* mosquitoes. For example, in coastal Tanzania, used tires and disposed containers were identified as commonest aquatic habitats for *Ae. aegypti* [11,20]. However, less is known regarding the ecology of these vectors in inland Tanzania, including small towns and rural settings. This is important to understand distribution of the vectors across the country, but more importantly to prevent introduction or spread of *Aedes*-borne arboviruses. To ensure effective control, such ecological studies should be complemented with investigations on susceptibility to commonly-used public health insecticides [21,22].

This study was therefore conducted to fulfil three key objectives: a) investigate spatial distribution of *Ae. aegypti* in Ifakara town and surrounding wards in south-eastern Tanzania, b) characterize aquatic habitats of the mosquitoes in the area, and c) assess susceptibility of the mosquitoes to insecticides commonly used for vector control.

Methods

Study area

Surveys for *Aedes* immatures were conducted in Ifakara town and surrounding wards, namely, Lipangalala (–8.16428, 36.68964), Viwanja Sitini (–8.13512, 36.68413), Mlabani (–8.13952, 36.68964) and Katindiuka (–8.13154, 36.71165), all in the Kilombero valley in south-eastern Tanzania (Figure1). Ifakara town and Viwanja Sitini are characterized as urban, while the other three are rural. The area has an average of 270m altitude, annual rainfall of 1200 - 1800mm, relative humidity of 51% - 71%, and daily temperatures of 20°C - 32.6°C [23]. The area experiences short rains in November and December, which is interrupted by dry months from January to March. Heavy rains continue from April to May or June, followed by dry July and September. It is a rapidly growing area with total population now estimated at 67,500, based on the 2.7% annual growth from the last census in 2012 [24].

Selection of sampling sites

The study area was divided into grids measuring 200m × 200m, in ArcGIS 10.4 environment (ESRI, USA) as previously done by Mwangungulu *et al.*, [25], and each grid assigned a unique identifier (Figure 2). We overlaid the grids with household geo-location data initially collected by Ifakara Health Institute's Health and Demographic Surveillance System [26]. This data was updated using population density maps from Google satellite imagery, and a high resolution settlement layer (HRSL) obtained from the Facebook Connectivity Lab and Centre for International Earth Science Information Network (CIESIN) [27].

From each ward, 34 grids containing human habitation or other actively-used buildings were selected as search grids. For each search grid, houses or buildings nearest to the centroid were identified as starting points for *Aedes* habitat searches. Where no informed consent was obtained, the next nearest consenting household was selected. From the starting points, we searched all potential aquatic *Aedes* habitats within 100m radii, visiting each search grid twice in dry season and twice in rainy season. We also mapped important features such as schools, marketplaces, worship areas, health facilities and water pumps using handheld GPS receivers (Magelan eXplorist GC, USA).

Sampling of mosquito immatures and characterization of their aquatic habitats

Sampling for *Aedes* immatures and characterization of their habitats was focused on natural and artificial water-holding objects such as tree holes, used tires, wells and discarded containers and animal feeding containers. Others included coconut shells, tarpaulins, broken grasses and other small objects that could potentially hold water longer than three days. All sites with *Ae. aegypti* larvae or pupae were geo-referenced using handheld GPS. The habitats were characterized by: a) location, b) size, c) apparent water color, d) presence of vegetation, e) presence of shading f) source of water in the habitat, g) whether the habitat was movable or not, and h) environmental and social activities surrounding the habitats.

We sampled larvae and pupae from each of the identified habitats using standard 350ml dippers, or a smaller 70ml dipper in cases where habitats were too small to sample using the standard dipper. The larvae and pupae were placed in white trays for morphological identification, using pictorial keys created by US Centres for Disease Control [28]. They were then sorted, counted and data recorded by habitat type, location and survey instance.

Mosquito rearing and identification of emergent adults

The *Aedes* larvae were transferred to the vector biology laboratory (VectorSphere), at Ifakara Health Institute (IHI) for rearing, and eventual morphological identification of emergent adults. The larvae were fed on Tetramin® baby fish food, and maintained at temperatures of 26°C ± 2 °C and relative humidity of 82% ± 10%. Pupae were collected each morning, counted and transferred to netting cages (30 × 30 × 30cm). Emergent adults were identified under stereo microscopes using taxonomic keys for *Aedes* mosquitoes [28,29].

Bioassays for insecticide susceptibility tests

Bioassays were performed following WHO insecticide susceptibility test guidelines [30,31]. Female *Ae. aegypti*, 3–5 days old originating from each ward were tested against commonly used insecticides as follows: two pyrethroids (deltamethrin; 0.05% and permethrin; 0.75%), one organochloride (dieldrin; 4%), one organophosphate (pirimiphos-methyl; 0.25%) and one carbamate (bendiocarb; 0.1%). The two pyrethroids included both types I & II respectively. In each experiment 120–150 mosquitoes were used, such that there were 20–25 individuals per test. Untreated controls were included, and the mosquitoes were initially observed for 60 minutes to observe knockdown at 10, 15, 20, 30, 40, 50 and 60min. The exposed and non-exposed mosquitoes were then provided with 10% glucose, and maintained at $28.0\text{ }^{\circ}\text{C} \pm 1.0\text{ }^{\circ}\text{C}$ and $80\% \pm 10\%$ relative humidity, then overall mortality observed after 24 hours.

Measurements of mosquito wing lengths

We also assessed whether mosquitoes from different wards or habitat types varied in size, by assessing their wing lengths. Emergent adults were anaesthetized at -10°C . Wings were removed from male and female mosquitoes (one wing/mosquito). Drops of distilled water were used to fix the wings onto glass slides. Wing lengths were measured, as distance from the apical notch to the auxiliary margins, under stereo zoom microscope using a micrometer ruler.

Data analysis

Statistical analyses above were done in the open-source R statistical software, version 3.231 [32]. Descriptive analysis was done to compare larval densities in different wards and seasons. Densities obtained from the 70ml dipper were compared to those from the standard 350ml dipper and a correlation coefficient calculated across all collections. Using this coefficient, the densities assessed by small dipper were all converted into standard dipper, so that all subsequent analyses were done on the standard dipper.

Generalized Linear Models [33] following Poisson distributions for count data were used to model number of larvae collected per dipper as response variable against season and habitat type as fixed factors. Logistic regression was also used to assess associations between positivity of different habitat types for *Aedes larvae* (proportion of individual habitats of one type that were positive with *Aedes larvae*). The relative risk (RR), odds ratios (OR) and their 95% CI were estimated. The *dabestr* package was used to assess mean differences of larval abundance between wards and seasons.

Larval indices, namely Container Index (proportion of containers infested with *Ae. aegypti* larvae or pupae), House Index (proportion of houses infested with *Ae. aegypti* larvae or pupae) and Breteau Index (number of infested containers per 100 houses) were also calculated by ward and season [22,34]. Mosquito wing lengths were compared using one-way ANOVA, followed by Tukey's *post-hoc* tests to assess mean differences between-ward for both male and female mosquitoes. Susceptibility status of *Ae. aegypti* was computed according to WHO guidelines [30], log-probit analysis was used to compute mean duration at which 50% (KD_{50}) and 95% (KD_{95}) of the exposed mosquitoes were knocked down.

Spatial and seasonal distribution of *Aedes* immatures were analyzed by geostatistical in ArcGIS 10.4 (ESRI, USA). Inverse Distance Weighted (IDW) interpolation technique [35,36] was used to visualize the areas with high larval densities. Representation of IDW maps show patterns based on the distance from one observed point to another. Known values (number of larvae) were used as key input feature to estimate unknown locations within 400m range based on estimated average flight range of *Aedes* mosquitoes [37,38]. Geo-processing extents and masks were defined to match the study area.

Results

Larval indices

A total of 1,515 breeding sites were visited in the dry season and 1,933 in rainy season. Of these, 286 (18.87%) in dry season and 283 (14.64%) in rainy season were positive with *Aedes* immatures. The proportion of infestation varied across wards and seasons as summarized in Table 1. In the dry season, high Container Indices (CI) were observed in Katindiuka, Viwanja Sitini and Ifakara Town wards, while in rainy season, high CIs were in Ifakara town, Viwanja sitini and Lipangalala wards. With regard to House Indices (HI), 2,315 and 2,832 houses were visited in dry and rainy season surveys, of which, 114 (4.9%) and 186 (6.6%) had at least one positive habitat respectively. Lipangalala ward had the highest HI during the dry season, while Ifakara town had highest HI in rainy season. Compared to dry season, HI increased during rainy season in all wards except Lipangalala (Table 1). It was also observed that Viwanja Sitini ward had highest Breteau Index (BI) in both seasons.

Densities of *Ae. aegypti* immatures, their distribution, and their aquatic habitats

A total of 63,470 larvae or pupae were collected from all wards. Of these, 76.3% (n = 48,459) were *Ae. aegypti*, 20.9% (n = 13,253) were *Culex* and 2.8% (n = 1,758) were identified as other *Aedes* species mosquitoes. In the dry season surveys, Ifakara town produced nearly one third of all immature *Aedes* and more than one third of immature *Culex*. In the rainy season however, Viwanja Sitini had more than one third of the *Aedes* immatures, while Katindiuka produced more than half of all *Culex*. Most *Culex* were found in dry season, while *Aedes* were more prevalent in wet season (Table 2).

Overall, most *Aedes* larvae were from used tires and clay pots followed by other containers such as discarded tins, buckets, drums and animal feeding pots (Figure 3). However, coconut tree holes and flower pots had far higher numbers of larvae per dip compared to all other habitat types, in the dry season (Table 3). Likelihood of getting larvae in individual tree holes was three times higher than in used tires (RR = 3.00 [2.58–3.50], P<0.01). However, in the rainy season, higher larval densities were observed in other habitats (Table 3).

Positivity of different habitat types for *Aedes* immatures

Positivity of the habitats for *Aedes* are summarized in Table 4. By assessing proportions for each type of habitat, it was determined that used tires were the most commonly infested with *Ae. aegypti* (89% positivity),

followed by containers (86% positivity) and clay pots (82% positivity), garage pits (64% positivity) and others (90% positivity). Majority of the positive breeding sites were movable, associated with human activities, or were found in and around residential areas, commercial places and garages. We also observed significantly higher *Aedes* positivity in rainy season than dry season. Also, number of positive habitats were higher if they had clear water than turbid water.

Spatial and seasonal distribution of *Aedes* immatures

The spatial distribution of *Aedes* immatures varied between dry and rainy season (Figure 4). In dry season, the highest infestation was from the center of Ifakara town toward western parts of Katindiuka ward. In the rainy season on the other hand, most infested locations were in southern Lipangalala and in Viwanja sitini (Figure 4).

Generally, fewer breeding sites were observed in dry season compared to rainy season in all study sites, though actual abundance varied significantly between sites. Ifakara town consistently had higher mean number of larvae than the other wards across seasons (Figure 5). We also estimated the residual mean differences of larval abundance between study ward.

Susceptibility of adult *Aedes aegypti* mosquitoes to insecticides

Ae. aegypti females were generally susceptible to all four classes of insecticides. Only in few instances did *Ae. aegypti* show reduced susceptibility to carbamates, and pyrethroids (Figure 6). Confirmed resistance was detected against only bendiocarb in the rainy season tests (Figure 6).

Overall knockdown KDT_{50} and KDT_{95} ranged from 7 to 112 minutes and 13 to 159 minutes respectively (Table 5). The knock down analysis revealed spatial and seasonal variation. Dieldrin and pirimiphos-methyl consistently achieved slower knock-down across wards, while bendiocarb and deltamethrin had quick knock-down. Knock-down times were not predictive of overall 24hr mortality. Often, mosquitoes were not affected by the insecticides during first 60 mins but mortality after 24 hours was still high.

Wing lengths of adult *Aedes aegypti* mosquitoes

Wing lengths, used here as a proxy for adult sizes of male and female *Ae. aegypti* ranged from 1.9mm to 3.5mm (Figure 7). The mean wing sizes were 2.48 (± 0.15) for mosquitoes from Ifakara town, 2.68 (± 0.23) in Katindiuka, 2.73 (± 0.20) in Lipangalala, 2.33 (± 0.18) in Mlabani and 2.68 (± 0.13) in Viwanja sitini. There was a significant difference in female mosquito's wing sizes across wards (ANOVA: F-statistic: 45.5 df = 4, $p < 0.001$). *Post hoc* analysis also revealed differences between pairs of wards (Figure 7). Also, the mean wing length of female *Ae. aegypti* were generally larger than those of male *Ae. aegypti* (ANOVA: F-statistic: 365.9 df = 1, $p < 0.001$).

Discussion

In Tanzania, majority of studies conducted on arbovirus vectors are in response to outbreaks, and are often concentrated in large urban areas [11]. Basic ecological studies to understand distribution and behaviors of the vectors, as well as their responses to interventions remain very few. This current study involved an exploratory survey of *Ae. aegypti* mosquitoes in a small town and its surrounding wards in south-eastern Tanzania. The findings therefore constitute essential baseline data on *Aedes* mosquitoes in this area where no outbreak has previously been reported, yet the risk is high. Given that there have been reports of arboviral infections such as Dengue and Chikungunya in neighboring districts [7], it is crucial to invest on studies to improve our understanding of the ecology of the vectors, so as to boost control options.

This study therefore assessed three important aspects, namely: a) spatial distribution of *Ae. aegypti* mosquitoes in Ifakara town and its surrounding wards in south-eastern Tanzania, b) characteristics of key aquatic breeding habitats of these mosquitoes, and c) the susceptibility of the mosquitoes to insecticides commonly used for vector control.

The main finding was that, larval indices (container index (CI), house index (HI) and breteau index (BI)) are high enough to signal significant risk of *Aedes*-borne diseases in the area. In the rainy season in particular, house and container indices in all wards exceeded the value of 5.0, specified by WHO for actionable arboviral infections risk [39–41]. Dry season risk was however confined to fewer wards though not completely absent from the rest of the wards. Immature *Ae. aegypti* infestation varied between wards and seasons, but remained significant even in dry season. This is expected since *Aedes* mosquitoes typically breed in man-made containers not fully dependent on rainfall. Besides, the vectors have fewer options of breeding sites in dry season hence elevating container level of infestation with immature *Ae. aegypti* (Table 1). On the contrary, aquatic habitats were relatively large in number during the rainy season, resulting in lower positivity rates (Table 1). This higher level of container infestation in the dry season concur with the study conducted in northern regions of Ghana which showed that, indices in the dry season was aggravated by poor water supply system in the area. As a result, facilitated the storing of water in pots and barrels for a period enough to bred *Aedes* mosquitoes [42].

We noted that *Ae. aegypti* prefers breeding in clean and stagnant waters. Similar to other studies [19,20,43]. Common habitats for *Ae. aegypti* were used tires, clay pots, flower pots, containers, coconut tree holes, pits, and on rare occasion disposed shoes, cooking pans, broken grasses and tarpaulins. Majority of these habitats were easy to discard, indicating an opportunity for proper waste management and environmental management as effective options for *Aedes* control, especially if used alongside traditional larviciding. As already highlighted by several previous studies, tires in particular serve as important breeding sites for *Ae. aegypti* because they can hold water for long periods even in dry season [11,19,44]. The multiple applications of used tires in the area will however complicate efforts to effectively dispose of the tires. For example, we observed that people use these tires as make-shift chairs, for playing by kids, for planting trees (residents believed that tires prevent plant pests) and for vehicle repairs.

A major natural breeding site in the area was coconut trees, which had artificial holes created for climbing during the coconut harvesting period. These holes served as the perfect breeding sites for *Ae. aegypti* mosquitoes. We recommend that coconut tree holes be filled with sands to prevent rainwater from stagnating [22]. Clay pots were also common in Katindiuka and Lipangalala wards where they were mostly used for collecting rainwaters for various domestic purposes. Unfortunately, residents did not know these pots bred

mosquitoes. We also observed rare habitats such as disposed coconut shells, broken glass, animal feeding containers, tarpaulins and discarded plastic shoes which produced high larval abundance (larvae/dipper). Higher abundance was influenced by size of habitats and the volume of water present breeding sites.

During data collection period, we raised awareness in surrounding communities about mosquito breeding behaviors and diseases they transmit. This led to a better understanding for them, and greater engagement of the communities in our work. Some breeding sites observed during the first visit were not there during subsequent visit as people became aware of the risks and hence proactively removed or covered potential habitats. This observation highlights the potential of educating communities about *Ae. aegypti* mosquito habitat sources and participatory control efforts. In Tanzania, the government is already implementing monthly clean-up campaigns, which could be leveraged to achieve such gains. Moreover, efforts to reduce mosquito population can prioritize areas identified with higher risk.

Mosquito sizes play an important role in overall vector competence, vectorial capacity and ability to disseminate viruses [45,46]. Smaller mosquitoes tend to have high contacts with hosts as they need more frequent blood meals than bigger mosquitoes, a phenomenon which could increase transmission [46]. In the other hand, bigger mosquitoes have been demonstrated to be more resistant toward insecticides [47]. Here, the wing length measurements for *Ae. aegypti* was done as previously documented by Nasci in 1986 [48], and showed a range of 1.9mm to 3.5mm. We also observed differences between administrative wards, though the extent to which such variations affect pathogen spread remains to be determined.

Lastly, we assessed how *Ae. aegypti* mosquitoes in the area would responds to control by commonly available insecticides. Fortunately, this study showed that the mosquito populations here are still generally susceptible to most insecticide classes except for bendiocarb against which there was resistance during the rainy season. Since this study is the first in the area of its kind, there are no immediate comparisons for the resistance profile. However, in studies done in Dar es salaam, Peru and Burkina Faso, resistance to pyrethroids and organophosphate was marked [20,49,50]. In our study, we have also observed notable spatial and seasonal variation toward Bendiocarb. Similar observation was previously documented for *Anopheles arabiensis* and *Culex pipiens* in south-eastern Tanzania [51,52]. Reduced susceptibility to pyrethroids observed in some of our assays, and the resistance seen against bendiocarb in the rainy season are however signs that we must remain vigilant as insecticide resistance could rapidly spread among the vector populations once active control programs begin. This would therefore mean that environmental management, including larval habitats search and removal, should be an important component of any anti-*Aedes* campaigns. As most habitats are those that can be discarded, combinations of insecticidal and non-insecticidal approaches would likely be effective.

Though the main objectives were successfully completed, this study also had various limitations. First, larvae and pupae were only collected in the selected grids (34 grids per ward) but these wards are not of the same surface area (Figure 2), thus some might have been underestimated. We therefore recommend the future studies should consider all the grids occupied by human habitations and building. Second, we adopted WHO standard dose specified for *Anopheles* mosquitoes, as we still do not have a comprehensive guideline for *Aedes*. However, some of these insecticides, such as pirimiphos methyl, permethrin and deltamethrin already have diagnostic concentrations specific for *Aedes* mosquitoes. Therefore, If the right concentration were used the results might have been different. For instance, results for permethrin (0.75%) demonstrated susceptibility toward standard concentration for *Anopheles*, which is three times the *Aedes* standard concentration (0.25%).

This mean *Aedes* mosquitoes might be resistant toward this concentration but susceptible toward *Anopheles* concentration. We recommend therefore that future studies should incorporate appropriate guidelines for the species.

Conclusion

This is the first study on ecology and insecticide susceptibility of *Aedes* mosquitoes in this area, and will provide a basis for future evaluation of its role in pathogen transmission, as well as options for its control. Infestation levels observed indicate that immediate actions should be taken to prevent outbreaks. The larval indices (container index, house index and breteaux index) are high enough to signal significant risk of *Aedes*-borne diseases in the area. Fortunately, the *Ae. aegypti* in the area are still susceptible to majority of insecticides used in public health, indicating available opportunities to include insecticides in the control programs. Since most habitats were those that can be discarded, integrating concepts of environmental management, insecticide use and community engagement could yield significant progress. While used tires, discarded containers and flower pots are key habitats for *Aedes* in the area, this study also identified coconut harvesting as an important risk factor, and the associated tree-holes as vital targets for *Aedes* control.

List Of Abbreviations

ANOVA - Analysis of variance

BI - Breteaux Index

CI - Container Index

CIESIN - Centre for International Earth Science Information Network

ESRI, USA - Environmental Systems Research Institute, United State of America

GPS—Geographical Position System

GVCR - Global Vector Control Response

HI - House Index

HRSL - High Resolution Settlement Layer

IDW - Inverse Distance Weighted

IHI - Ifakara Health Institute

KDT—Knock Down Time

MRCC - Medical Research Coordinating Committee

NIMR - National Institutes of Medical Research

OR - Odds Ratios

RR - Relative Risk

WHO - World Health Organization

Declarations

Author's contribution

NFK was involved in conceptualization and designing of the study, data collection, data entry and formal analysis, conducting bioassay tests, producing maps and writing the manuscript. FOO was involved in conceptualization, study design, supervision and manuscript revision. HSN was involved in analysis, reviewing and supervision. AJL, SAM and EWK were involved in study design and producing maps, BJM and DSM were involved in data collection and reviewing.

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Ethics approval and consent to participate

Approval for this study was obtained from institutional review board of Ifakara Health Institute (Ref: IHI/IRB/No: 10-2017), and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR), (Ref: NIMR/HQ/R.8a/Vol. IX/2555). Meetings with local leaders were held to highlight objectives, benefits and risks associated with the study. Informed consent was obtained from all owners of property around which the mosquito surveys were conducted.

Consent for publication

Approval for publishing this paper was obtained from National Institutes of Medical Research (NIMR), (Ref: NIMR/HQ/P.12 Vol. XXIX/9).

Competing interests

Authors have no competing interests to declare.

Availability of data and materials

All data for this study will be available when requested to the lead author.

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Tables

Table 1: Summary of *Ae. aegypti* larval survey indices by ward and seasons. The data is presented for the different indices separately

Wards	Dry season			Rainy season		
	CI (%)	HI (%)	BI (%)	CI (%)	HI (%)	BI (%)
Ifakara town	21.4	4.18	16.74	27.4	7.12	9.54
Katindiuka	18.7	4.45	9.37	11.2	6.78	7.22
Viwanja sitini	29.5	6.67	20.28	26.2	6.75	15.25
Mlabani	13.01	3.33	5.12	11.9	6.58	10.53
Lipangalala	21.4	6.44	8.44	19.6	5.11	11.11

CI (container index: ratio of larval infested to total inspected containers); HI (House index: ratio of larval infested to all inspected houses); BI (Breteau index: ratio of positive containers per 100 houses inspected).

Table 2: Sampled populations of *Aedes* and *Culex* larvae collected in all aquatic habitats in Ifakara town and its surrounding wards.

Wards	Dry season				Rainy season				Total	
	<i>Aedes</i>		<i>Culex</i>		<i>Aedes</i>		<i>Culex</i> larvae		<i>Aedes</i>	<i>Culex</i>
	N	%	N	%	N	%	N	%	N	N
Ifakara town	5325	32	4217	39	6769	20	0	0	12094	4217
Katindiuka	2845	17	1240	11	2383	7	919	37	5228	2159
Viwanja sitini	3527	21	3116	29	11652	35	0	0	15179	3116
Mlabani	1833	11	826	8	7698	23	15	1	9531	841
Lipangalala	3284	20	1386	13	4901	15	1534	62	8185	2920

N denotes number of larvae collected, % denotes percentage of larvae by ward

Table 3: Larval densities in different aquatic habitats of *Ae. aegypti* mosquitoes in the dry and rainy seasons in the study area

Habitat type	Larvae (N)	Habitats (n)	Mean (95% CI)	RR (95% CI)	P- Value
Dry season					
Used tire	844	51	16.5 (15.46-17.70)	1	
Clay pot	652	44	14.8 (13.7-16)	0.89 (0.80-0.99)	0.034
Container	93	24	3.9 (3.16-4.75)	0.23 (0.19-0.29)	<0.01
Flower pot	163	9	18.1 (15.53-21.12)	1.09 (0.93-1.29)	0.292
Pit	96	7	13.7 (11.23-16.75)	0.83 (0.67-1.02)	0.081
Tree hole	199	4	49.8 (43.3-57.17)	3 (2.58-3.50)	<0.01
Others	12	6	2 (1.14-3.52)	0.12 (0.07-0.21)	<0.01
Rainy season					
Used tire	1276	55	23.2 (21.96-24.51)	1	
Clay pot	978	55	17.8 (16.7-18.93)	0.77 (0.70-0.83)	<0.01
Container	504	27	18.7 (17.11-20.37)	0.80 (0.72-0.89)	<0.01
Flower pot	273	17	16.1 (14.26-18.01)	0.69 (0.61-0.79)	<0.01
Pit	133	7	19 (16.03-22.52)	0.82 (0.69-0.98)	0.028
Tree hole	68	4	17 (13.4-21.56)	0.73 (0.57-0.94)	0.012
Others	119	5	23.8 (19.87-28.48)	1.03 (0.85-1.24)	0.79

RR = risk ratio, CI = confidence interval, N = total number of larvae/dips, n = number of habitats. Category used as reference R = 1, means reported here are predicted from generalized linear model which is average of larvae per dipper to number of breeding sites. Used tire was selected as reference because they were present in all study sites. "Others" included positive breeding sites such as disposed shoes, coconut shells, tarpaulins, broken glasses and open plastic bottles.

Table 4. Results of the logistic regression analysis showing positivity and negativity of habitats of different characteristics for immature *Ae. aegypti* mosquitoes.

Parameter	Categories	N (%)			Univariate		Multivariate	
		Positive	Negative	Total	OR (95%CI)	P-Value	OR (95%CI)	P-Value
Habitat type	Used tires	89 (84)	17 (16)	106	1		1	
	Clay pot	81 (82)	18 (18)	99	0.86 (0.42-1.78)	0.68	0.55 (0.21-1.42)	0.216
	Container	44 (86)	7 (14)	51	1.2 (0.46-3.11)	0.70	1.07 (0.33-3.48)	0.904
	Flowerpot	22 (85)	4 (15)	26	1.05 (0.32-3.44)	0.93	0.62 (0.13-2.95)	0.551
	Pits	9 (64)	5 (36)	14	0.34 (0.10-1.15)	0.08	0.11 (0.01-2.54)	0.172
	Tree hole	7 (88)	1 (12)	8	1.34 (0.15-11.58)	0.79	0.92 (0.03-31.86)	0.962
	Others	10 (90)	1 (9)	11	1.91 (0.23-15.91)	0.55	2.98 (0.26-34.82)	0.383
Size	Large	36 (71)	15 (29)	51	1		1	
	Medium	129 (84)	25 (16)	154	2.15 (1.03-4.5)	0.042	1.73 (0.71-4.18)	0.2165
	Small	97 (88)	13 (12)	110	3.10 (1.35-7.17)	<0.001	0.98 (0.33-2.89)	0.966
Season	Dry season	97 (67)	48 (33)	145	1		1	
	Rainy season	165 (97)	5 (3)	170	16.3 (6.3-42.4)	<0.001	19.73 (6.61-58.94)	<0.001
Movability	Immovable	20 (74)	7 (26)	27	1		1	
	Movable	242 (84)	46 (16)	288	1.8 (0.74-4.6)	0.192	0.36 (0.03-5.24)	0.46
Turbidity	Clear	145 (88)	20 (12)	165	1		1	
	Turbid	109 (83)	22 (17)	131	0.68 (0.36-1.32)	0.254	0.79 (0.38-1.67)	0.5417
	Very turbid	8 (42)	11 (58)	19	0.10 (0.03-0.27)	<0.001	0.13 (0.04-0.44)	<0.001
Shades	Full	115 (86)	19 (14)	134	1		1	

	Partial	126 (81)	29 (19)	155	0.72 (0.38- 1.35)	0.303	0.74 (0.35- 1.60)	0.45
	None	21 (81)	5 (19)	26	0.7 (0.23- 2.06)	0.511	0.43 (0.09- 1.92)	0.27
Water source	Domestic	12 (63)	7 (36)	19	1			
	Rainwater	250(84)	46 (16)	296	3.17 (1.19- 8.45)	0.02	1.11 (0.28- 4.39)	0.87

OR=odds ratio, CI=confidence interval, N=number of breeding sites, Category used as reference R=1, social and environmental factors were dropped in the analysis they had less impact.

Table 5 Knock-down times of *Ae. aegypti* mosquitoes collected from different sites

Insecticide	Ward	Dry season		Rain season	
		KDT ₅₀ ±SE (min)	KDT ₉₅ ±SE (min)	KDT ₅₀ ±SE (min)	KDT ₉₅ ±SE (min)
Bendiocarb	Ifakara town	21.44 ± 4.52	28.68 ± 8.95	14.58 ± 6.28	30.26 ± 12.90
	Katindiuka	16.89 ± 3.05	22.15 ± 6.17	22.85 ± 6.68	39.34 ± 13.45
	Lipangalala	30 ± 5.82	41.16 ± 10.29	32.94 ± 8.04	53.86 ± 15.43
	Mlabani	25.13 ± 6.94	42.28 ± 13.66	30.77 ± 6.48	44.18 ± 11.70
	Viwanja sitini	28.91 ± 5.67	38.99 ± 10.08	39.77 ± 9.18	63.91 ± 18.76
Deltamethrin	Ifakara town	9.67 ± 3.56	14.11 ± 5.78	6.19 ± 5.9	17.16 ± 8.83
	Katindiuka	11.45 ± 4.42	19.95 ± 7.65	12 ± 9.60	37.44 ± 18.07
	Lipangalala	29.09 ± 46.16	31.59 ± 76.50	12.46 ± 3.44	18.52 ± 6.27
	Mlabani	7.2 ± 4.58	12.30 ± 5.27	16.41 ± 13.99	59.19 ± 30.42
	Viwanja sitini	7.12 ± 4.48	13.08 ± 5.75	17.64 ± 5.45	30.20 ± 11.55
Dieldrin	Ifakara town	36.02 ± 7.32	52.69 ± 13.26	75.43 ± 49.31	101.68 ± 103.19
	Katindiuka	40.73 ± 7.56	57.80 ± 13.96	22.9 ± 8.44	47.57 ± 17.40
	Lipangalala	43.32 ± 5.95	53.86 ± 10.68	85.57 ± 70.37	146.57 ± 154.15
	Mlabani	70.9 ± 33.93	102.46 ± 75.05	40.21 ± 8.70	62.23 ± 17.21
	Viwanja sitini	49.01 ± 6.89	62.59 ± 13.51	66.17 ± 370.887	70.70 ± 620.79
Permethrin	Ifakara town	12.69 ± 7.55	32.13 ± 14.93	7.2 ± 7.59	23.42 ± 12.58
	Katindiuka	-	-	10.56 ± 8.14	30.66 ± 15.25
	Lipangalala	8.52 ± 4.38	14.87 ± 5.95	12.28 ± 2.60	16.27 ± 4.68
	Mlabani	29.83 ± 7.21	47.19 ± 13.58	9.54 ± 8.73	30.60 ± 15.78
	Viwanja sitini	15.38 ± 4.41	24.73 ± 9.57	18.28 ± 3.17	23.45 ± 7.01
Pirimiphos-methyl	Ifakara town	75.66 ± 44.78	109.97 ± 95.41	71.03 ± 37.01	114.39 ± 83.36
	Katindiuka	78.03 ± 50.32	125.04 ± 109	26.66 ± 7.90	48.30 ± 15.75
	Lipangalala	79.14 ± 52.26	123.36 ± 111.14	32.36 ± 10.12	63.19 ± 22.59

Mlabani	60 ± 15.83	84.06 ± 38.22	43.72 ± 8.41	63.61 ± 16.69
Viwanja sitini	83.29 ± 102.4	108.97 ± 193.88	39.75 ± 14.95	84.60 ± 41.95

N number of tested mosquitoes, SE standard error, KDT_{50} time taken for 50% of the tested mosquitoes to be knock-down, KDT_{95} time taken for 95% of the tested mosquitoes to be knock-down. In each experiment there were six replicates and 120-150 *Aedes* female mosquitoes.

Figures

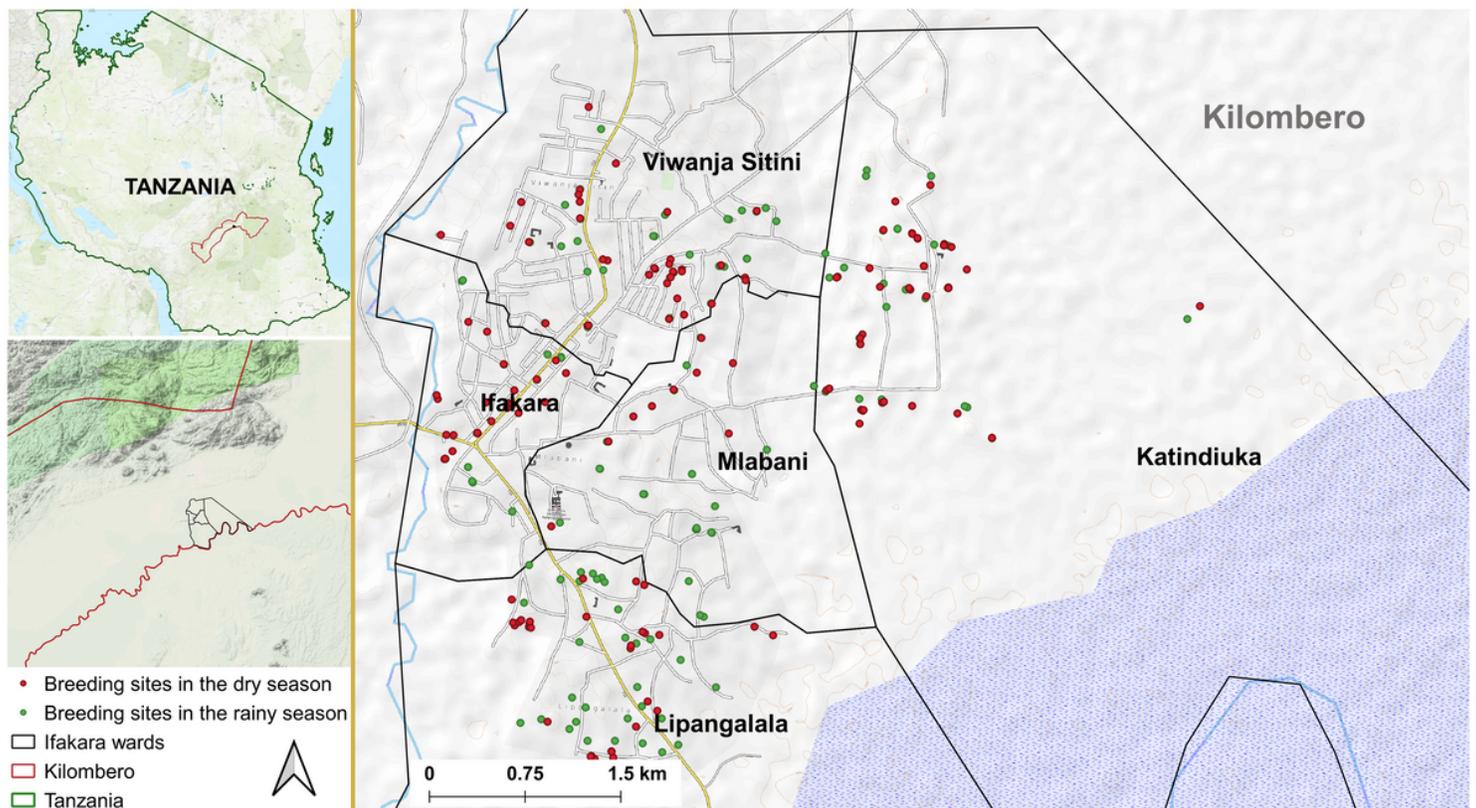


Figure 1

Study area: map Ifakara town and its surrounding wards showing locations where *Ae. aegypti* immatures were sampled in dry and rainy seasons.

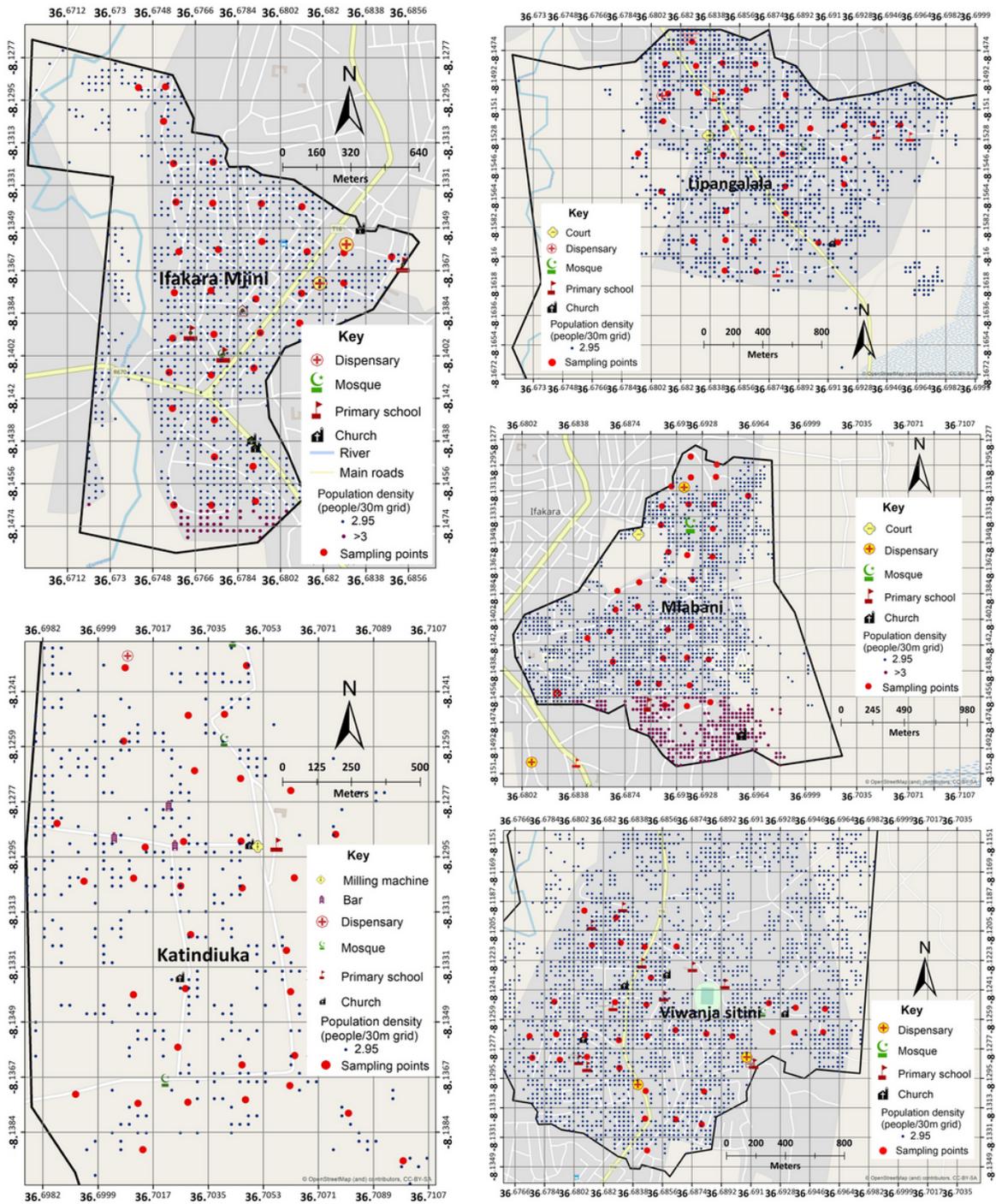


Figure 2

Selected grids in the study area, which were sampled for conducting *Ae. aegypti* larval surveys in dry and rainy seasons. Estimated population densities are also shown.



Figure 3

Various breeding sites identified in the study area: A) used vehicle tires, here repurposed by residents as seats B) used tires kept for protecting trees from pests, C) disposed coconut shells, D) flower pots, E) animal feeding container, F) broken grasses, G) disposed containers, H) coconut tree holes, I) clay pots, J) small containers, and J) pits such as those at construction sites, in garages, or inspection chambers in waterworks.

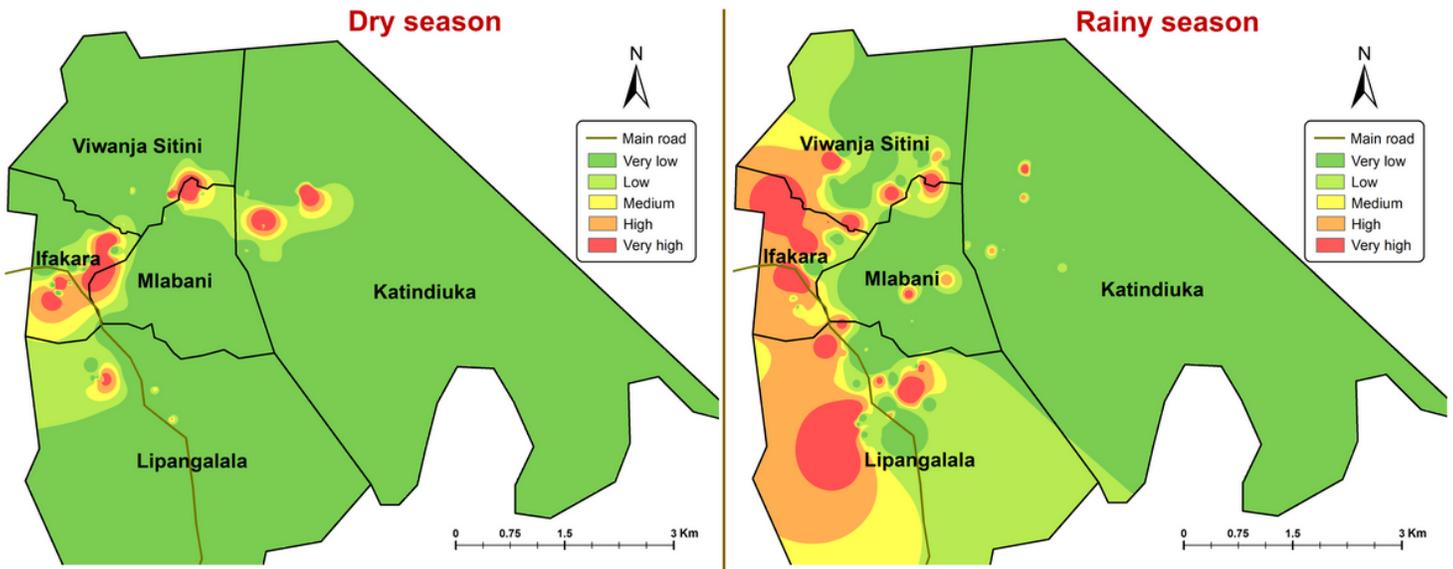


Figure 4

Spatial and seasonal distribution of *Aedes* larvae infested locations. Very Low: 0-16 *Aedes* larvae/dip; Low: 17-20 larvae/dip; Medium: 21-23 larvae/dip; High: 24-28 larvae/dip; Very High: 29-37 larvae/dip.

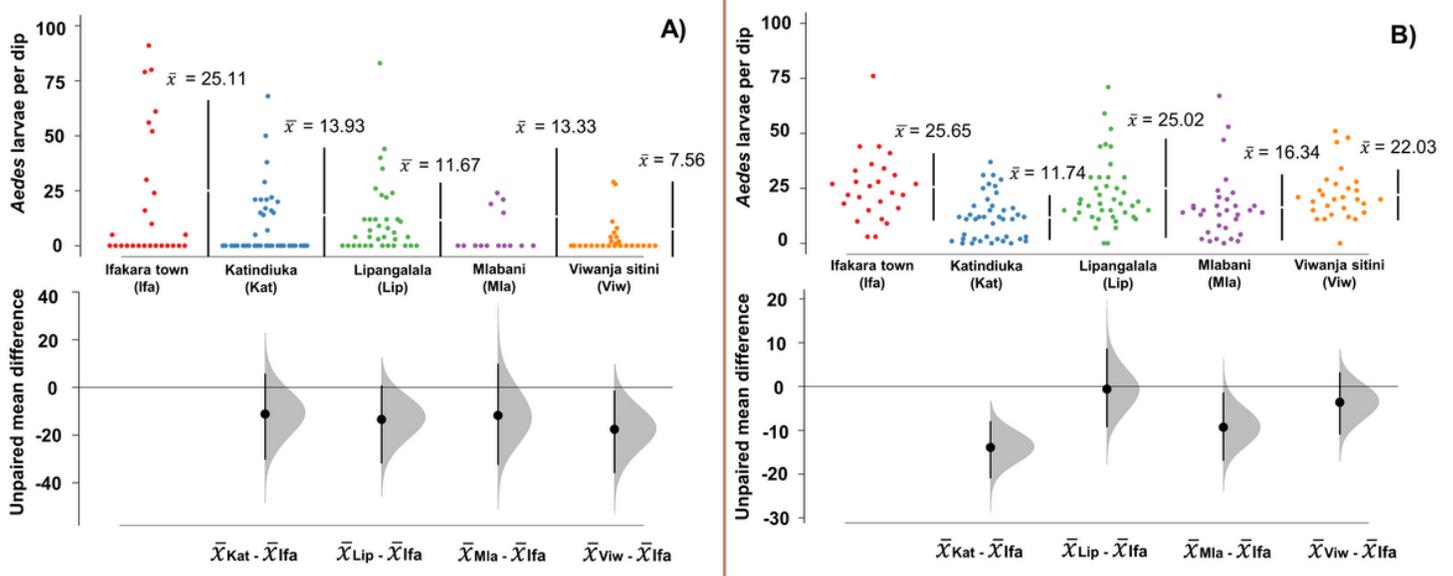


Figure 5

Estimated means of *Aedes* larvae/dip in Ifakara town and surrounding wards in: A) Dry season, and B) Rainy season. Estimation plots are used to portray the distribution of residual mean differences of larval abundance between study wards. The vertical lines represent mean \pm confidence levels (the gap in the line is the mean). The filled curves indicate the resampled mean difference distribution of the larval abundances with reference to Ifakara town. Black vertical line indicates 95% confidence level. Black dot indicates mean difference to the reference group. The significance is considered depending on how far the means of residual deviated from the reference line.

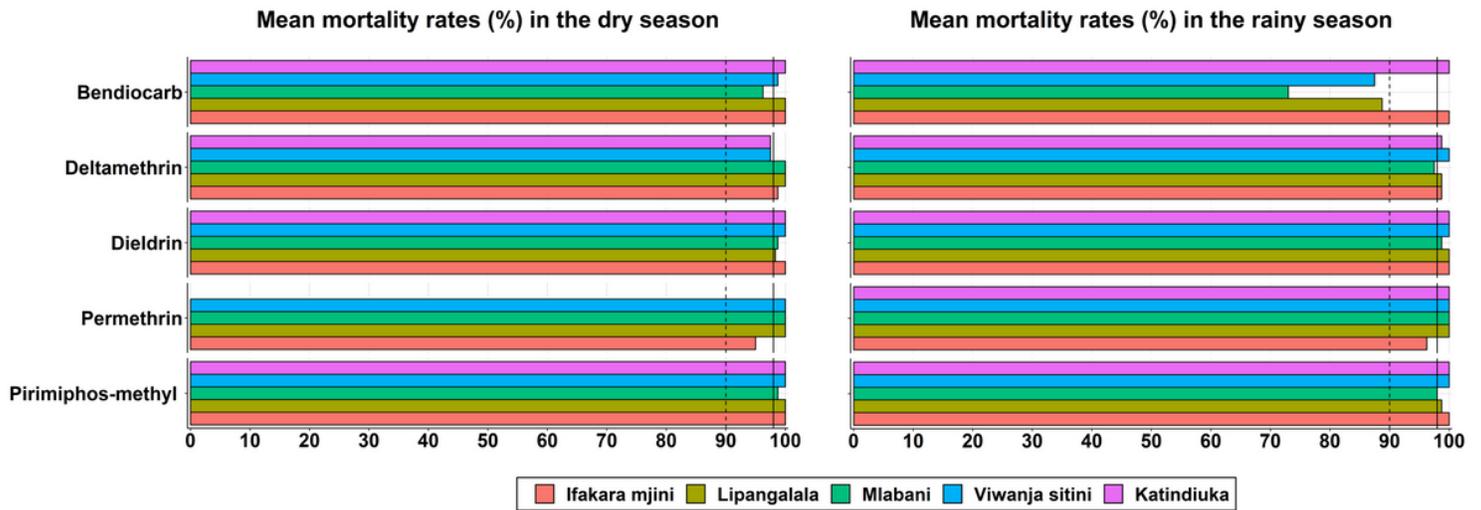


Figure 6

Mean mortality demonstrating susceptibility status of *Ae. aegypti* in dry and rainy seasons. The solid lines ($\geq 98\%$ mortality) indicate that mosquitoes are fully susceptible to insecticide, while the dotted lines (90-98% mortality) indicate possible resistance requiring confirmation.

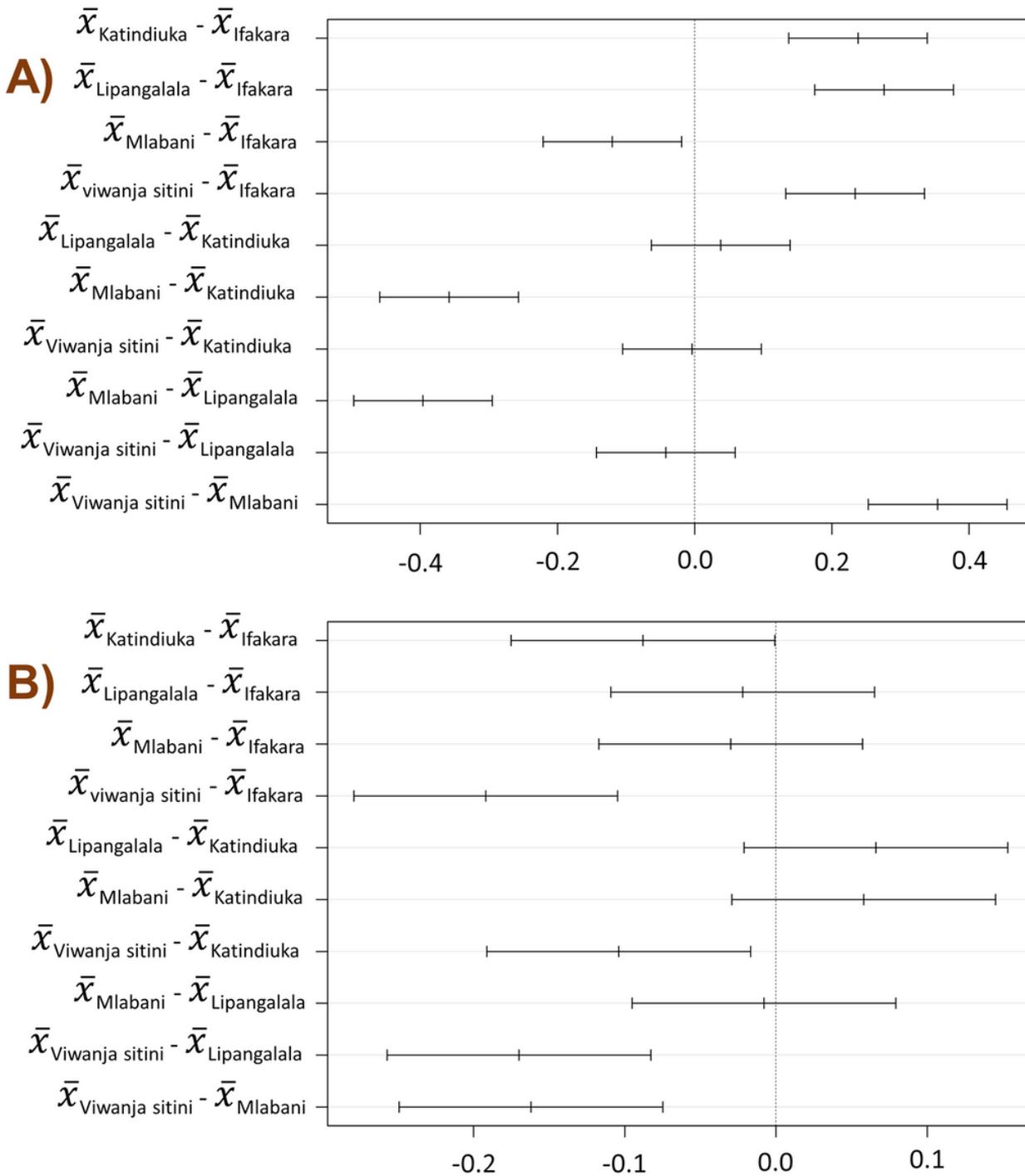


Figure 7

Differences in mean wing lengths between wards. Pairwise comparisons are shown at 95% Confidence Levels for A) Female and B) Male *Aedes aegypti* mosquitoes.