# Aquatic habitats of the malaria vector, Anopheles funestus in rural south-eastern Tanzania

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# Abstract

Background In rural south-eastern Tanzania, Anopheles funestus now dominates malaria transmission, mediating nearly nine in every ten new malaria infections. However, little is known about the ecological requirements and survival strategies of this mosquito species in the wild. Methods Potential mosquito aquatic habitats were systematically searched along 1000 m transects radiating from the centers of six villages in south-eastern Tanzania. All water bodies were geo-referenced, characterized and examined for presence of Anopheles larvae using standard 350mls dippers or 10L buckets. Larvae were collected for rearing, and the emergent adults identified to generic or species level, to confirm habitats containing An. funestus . Results One hundred and eleven (111) habitats were identified and assessed from the first five villages (all <300m altitude). Of these, 36 (32.4%) had An. funestus larvae co-occurring with other mosquito species. Another 47 (42.3%) had other Anopheles species and/or culicines but not An. funestus , and 28 (25.2%) had no mosquitoes. There were three main habitat types occupied by An. funestus, namely: a) small spring-fed pools with well-defined perimeters (36.1%), b) medium-sized natural ponds retaining water most of the year (16.7%), and c) slow-moving waters along river tributaries (47.2%). The habitats generally had clear waters with emergent surface vegetation, depths > 0.5m and distances < 100m from human dwellings. They were permanent or semi-permanent, retaining water most of the year. Water temperatures ranged from 25.2 to 28.8°C, pH from 6.5 to 6.7, turbidity from 26.6 to 54.8 NTU and total dissolved solids from 60.5 to 80.3 mg/L. In the sixth village (altitude >400m), very high densities of An. funestus were found along rivers with slow-moving clear waters and emergent vegetation. Conclusion This study has documented the diversity and key characteristics of aquatic habitats of An. funestus in south-eastern Tanzania, and will form an important basis for further ecological studies towards improved control strategies. Given the observed characteristics, An. funestus habitats in the area can indeed be described as fixed, few and findable. Future studies should therefore investigate potential of targeting these habitats with larviciding or larval source management to complement malaria control efforts in areas dominated by this vector.


# Background

Anopheles funestus mosquitoes have been a major malaria vector in many east and southern African countries for several years [1–4]. In south-eastern Tanzania they consistently mediate more than 85% of all the malaria transmission across several villages [4–6]. Their dominance in pathogen transmission [4, 7] is attributable to factors such as: a) being predominantly anthropophilic (i.e. strong preference for blood from humans over other vertebrates) and endophilic (i.e. strong preference for biting and resting indoors than outdoors) [8, 9], b) their high resistance to commonly-used pyrethroid insecticides [10–14], and c) their superior daily survival probabilities as reflected in the higher parity rates compared to other Anopheles species [4, 5, 7].

The supremacy of An. funestus in malaria transmission has been observed even in areas where they occur at far lower densities compared to other malaria vectors such as Anopheles arabiensis [4, 5, 15]. In such settings, the infrequent occurrence partly explains why their behaviors are relatively under studied in the field. More generally, An. funestus are also far easier to find as adults than as larvae. As a result, this species rarely features in larval surveys of Anopheles species. Researchers therefore sometimes rely on adult collections rather than larval collections to obtain enough samples for insecticide resistance testing [4], which according to WHO protocols require specimen collected as larvae and with synchronized age groups [16].

It has previously been suggested that an in-depth ecological understanding, followed by improved targeting of An. funestus could potentially improve their control, and significantly reduce malaria transmission in areas where the vector dominates [4]. Given their strong resistance to currently available insecticides commonly applied on insecticide-treated nets (ITNs) and/or indoor residual spraying (IRS) [10–14], supplementary measures targeting the aquatic stages of the mosquitoes are critical for more effective control of An. funestus. This requires rigorous surveys to identify and characterize preferred larval habitats by the An. funestus [17]. Strategies such as larval source management and targeted larviciding could indeed significantly improve control efforts and accelerate progress towards malaria elimination, especially in communities where the aquatic habitats are fixed, few and findable [18, 19].

A previous study in western Kenya reported that An. funestus prefers to oviposit in large semi-permanent water bodies containing aquatic vegetation and algae [20]. A separate study in coastal Kenya observed these species breeding in vegetated aquatic habitats that are stable and permanent, and were along river streams [21]. In Cameroon, it was demonstrated that An. funestus habitats were often in open savannas instead of deep or degraded forests, had greater exposure to sunlight and high temperatures, and yielded emergent adults for much longer than other Anopheles, often peaking at the start of the dry season [22, 23]. Unfortunately, in south eastern Tanzania where the species now dominates transmission, there have not been detailed studies of its natural aquatic habitats and responses to interventions. This situation is complicated by researcher's inability to colonize the species inside laboratories, which would enable such studies.

This current baseline study was therefore aimed at identifying and characterizing the main larval habitats of An. funestus to advance knowledge of its aquatic ecology. The findings were expected to provide a basis for further investigations into improved control strategies targeting the species, and also to inform ongoing efforts for rearing this species under laboratory conditions.

## Materials And Methods

# Study areas

This study was conducted in six villages of Kilombero and Ulanga districts in south-eastern Tanzania (Fig. 1). Five of these villages were located at altitudes less than 300 m above sea level, while the sixth was at an altitude greater than 400 m. In Kilombero district the study villages were Ikwambi (-7.97927°S, 36.81630°E), Kisawasawa (-7.89657°S, 36.88058°E) and Mpofu (-8.17220°S, 36.21651°E), while in Ulanga district, the villages were Kilisa (-8.37544°S, 36.57355°E), Ruaha (-8.9063°S, 36.7194°E) and

Tulizamoyo (-8.35447°S, 36.70546°E). The study villages were selected based on high abundance of An. funestus mosquitoes following previous surveillance work done by Ifakara Health Institute. The annual rainfall and temperature ranges in these villages was 1200–1800 mm and 20-32.6 °C respectively. The main economic activities were crop farming (mostly rice and maize farming) and livestock keeping.

# Larvae collection and rearing

This study was done between January and September 2018, and repeated between October and December 2019. The study villages were surveyed for presence of aquatic habitats along transects of 1000 meters, each radiating from an approximated village centroid. All identified water bodies were marked, geo-referenced, physically characterized and examined for the presence of Anopheles larvae. Standard 350 ml dippers or 10 L plastic buckets were used to sample water from the pools (Fig. 2). When the water bodies consisted of rivers and streams, water was sampled from various parts along the river length over distances not exceeding 1000 meters. Parts of the rivers with or without Anopheles larvae were similarly characterized and geo-referenced. The buckets were used in sites where it was impractical to use the dippers (e.g. habitats with depths greater than 50 cm), and also to collect the larvae for further rearing and identification. The larvae that were collected from different aquatic sites were transported to the insectary at the Ifakara Health Institute for rearing to adults. Morphological identification was done to confirm different mosquito genera and species, and to document the presence or absence of An. funestus in the habitats sampled.

Once in the insectary, the larvae were kept in rearing pans (32 cm diameter and 5 L holding capacity) labeled with information on the dates and place of larvae collection. The temperature in the insectary was kept at 26 °C ± 2 °C and relative humidity at 82% ± 10%. The larvae were fed with Tetramin® fish food until they developed into pupae and emerged into adult mosquitoes. Emerging adult mosquitoes were collected using mouth aspirator, killed by freezing and identified using morphology-based identification keys developed by Gilles and Coetzee [9, 24]. All identified An. funestus mosquitoes were then packed individually in 1.5 ml Eppendorf tubes with silica gel and submitted to molecular laboratory for sibling species identification by polymerase chain reaction (PCR) assays as described by Koekemoer et al., [25]. Habitats positive for An. funestus were then identified among all the surveyed habitats.

# Characteristics of aquatic habitats

Characteristics of all the aquatic habitats as well as the surrounding environments were recorded. For the habitats, information collected included water movement, water color, presence or absence shade over habitat, habitat size (width and depth), vegetation type and amount, presence and amount of algae, water type and distance from the nearest homes.

Additionally, the physicochemical characteristics of water in the larval habitats were assessed in four of the six villages, namely Tulizamoyo, Ikwambi, Kisawasawa and Kilisa. Parameters assessed included: water temperature (degree celsius), pH (scale of 0–14), conductivity (Siemens/meter), total dissolved solids (mg/L) and turbidity (nephelometric turbidity units, using 2100Q portable turbidity meter). Assessments of these parameters were conducted in the field sites immediately after collection of larvae from the habitats. Lastly, nitrate levels (milligrams per liter) were also analyzed by spectrophotometric method. To do this, one liter of water samples from each habitat in the study sites was collected, stored in a cooler box and sent to the laboratory at Ifakara Health Institute for analysis within 24 hours post collection.

# Data analysis

Analysis was done using open source software, R programming language [26]. A total of 16 environmental variables were used to identify the main predictors for the presence of An. funestus larvae in the study villages. Univariate and multivariate logistic regression analyses were used to assess associations with individual predictors and the combined influence of multiple predictors on the occurrence of An. funestus larvae. Odds ratios and their 95% confidence intervals are reported, and the statistical differences were considered significant when p-values $< 0.05$.

## Results

# Occurrence of An. funestus and other mosquito species in different habitats

A total of 111 potential habitats were surveyed of which 83 (74.8%) were identified to have larvae while 28 (25.2%) did not have. Of the 83 larval habitats that were positive for mosquito larvae, 36 (43.4%) had An. funestus. More than two thirds of the An. funestus habitats (69.4%; n = 25) were shared with Culex mosquitoes, while one third (30.6%; n = 11) were shared with other Anopheles species. The An. funestus habitats included: spring-fed pools (36.1%; n = 13), medium-sized natural ponds (16.7%; n = 6) and river tributaries with slow moving waters (44.2%; n = 17).

Adult mosquitoes emerging from the same habitats as An. funestus included: other members of the An. funestus group (64%; n = 696), Culex mosquito species (24.5%; n = 267), An. coustani (6.2%; n = 67), members of the An. gambiae s.l, (4.3%; n = 47) and other species (1%; n = 11). PCR identification of the 501 An. funestus complex revealed that 53.3% (n = 267) were An. funestus s.s, 28.7% (n = 144) were Anopheles rivulorum, 11.8% (n = 59) were Anopheles leesoni and 6.2% (n = 31) were unidentified due to non-amplification in the PCR assays. The An. funestus s.s commonly shared habitats with the other sibling species including An. leesoni and An. rivulorum.

# Water type and vegetation

Table 1 summarizes different environmental variables in aquatic habitats associated with presence of An. funestus and other mosquito species. An. funestus preferred permanent and semi-permanent aquatic habitats characterized by stagnant or slow-moving waters. Presence of emergent vegetation was also strongly associated with the presence of An. funestus larvae ($P < 0.01$). Multivariate regression analysis showed that heavily shaded aquatic habitats, especially along the rivers also had high chance of harboring An. funestus larvae compared to others ($p < 0.05$). Habitats with water depths greater than 50 cm, located within 100 m distance from human dwellings had high likelihood of containing An. funestus larvae compared to habitats less than 50 cm and habitats far from homes, though the effect of these two attributes was not statistically significant (Table 2).

At higher altitudes, such as in Ruaha village, which was higher than 400 m above sea level, all the An. funestus larvae collected were from the rivers. The river sections acting as the breeding sites for An. funestus had slow-moving and clear waters near their banks. They were characterized by abundant emergent vegetation and water depths of greater than 50 cm (Fig. 3), and were within 100 m from human dwellings. The physical characteristics at these altitudes were the same as in the other habitats of An. funestus found below 300 m altitude, i.e. the natural perennial ponds, or small spring-fed water pools with well-defined areas (Fig. 4).

# Physicochemical characteristics of water in the aquatic habitats

Table 3 shows the median values of physicochemical parameters in larval habitats of different mosquito species. The pH in all An. funestus larval habitats was weakly acidic, ranging from 6.5 to 6.7. The concentration of total dissolved solids (tds) was highest in the water pools without any larvae (88.7–148.0 mg/L) and lowest in An. funestus habitats (60.5–80.3 mg/L). Turbidity was low in all habitats surveyed (11.5–64.0 NTU), while conductivity was higher in water pools without larvae (286.0 [99.2–310.0] μS/cm) compared to habitats containing An. funestus (151.0 [134.0-165.0] μS/cm) and others. The association between these physicochemical characteristics and the occurrence of An. funestus was however not statistically significant (Table 4).

## Discussion

Although An. funestus are among the most important vectors of malaria in Africa, little is known regarding their larval ecology and development. This crucial gap needs an urgent solution, but is perpetuated by the inability of most mosquito biologists to create laboratory colonies of this vector species. Understanding the basic environmental parameters that influence mosquitoes breeding and oviposition can improve the planning, development and deployment of new interventions to control malaria transmission [27]. This study identified and characterized larval habitats of An. funestus in south-eastern Tanzanian villages of Ulanga and Kilombero districts, where this mosquito species now mediates more than four fifths of ongoing malaria infections [4, 6].

The study examined more than 100 potential habitats across six villages, and identified three main habitat types. First were small water wells with well-defined edges and were spring fed, some of which were also used by locals as domestic water sources (Fig. 4b). These habitats were often occupied by multiple species of the An. funestus group, and in some cases, they were shaded by large trees. The second type of habitat was medium-sized ponds, for which the central part retained water for all or most of the year. These habitats often had surface vegetation (Fig. 4a) and were occupied by multiple other Anopheles species such as An. arabiensis. Third was the riverside habitats consisting of the slow-moving waters on the rivers or river tributaries, also with vegetation (Fig. 4c). These habitats were mostly found at altitudes above 400 m above sea level, unlike the other two habitats which were more common at lower altitudes below 300 m. In summary, An. funestus in this area appears to prefer permanent and semi-permanent aquatic habitats with stagnant or slow-moving waters, emergent vegetation e.g. algae on swamp surfaces, clear waters at depths exceeding 50 cm and nearness to human dwellings.

Recent entomological surveys have demonstrated that the lower altitude areas have higher An. arabiensis densities than An. funestus while the higher altitude areas have more An. funestus than An. arabiensis [5, 6, 28–30]. However, it has also been shown that An. funestus dominates malaria transmission across the valley in both the areas where its densities are lower and areas where its densities are higher than those of An. arabiensis [4, 6]. A separate epidemiological survey of human malaria infections has also demonstrated correlations between malaria parasite prevalence in all age groups and proportional abundance of An. funestus (Swai et al., unpublished). Villages with the highest densities An. funestus also had the highest malaria burden (Swai et al., unpublished). The data generated from this habitat characterization study is therefore particularly important in the ongoing efforts for malaria prevention and will form a basis for future control efforts.

The findings from this study that An. funestus prefer permanent or semi-permanent habitats characterized by emergent vegetation is concurrent with past evidence from earlier investigations in Kenya [20, 21, 31]. Although this current study did not assess seasonality of An. funestus larvae densities in the different habitats, the observed preference of permanent and semi-permanent water bodies explains the known seasonality of its adult densities in the same study villages as observed in recent entomological surveys [4, 30]. The adult densities of An. funestus tend to peak after the rains just before the dry seasons begin, and are sustained by the large permanent water bodies [20]. Although no detailed studies have been done in this area targeting An. funestus aquatic habitats, early accounts by Gillies and DeMeillon [9], as well as limited surveys done nearly fifty years ago in Ifakara area (which neighbors the current study site) already suggested an association between the late peaks in An. funestus densities and the large perennial habitats [32].

Although there was no clear statistical association, the An. funestus habitats had depths greater than 50 cm and were located within 100 m from the human dwellings. This is likely due to the anthropophagic nature of these mosquitoes [33], and further explains the importance of this species in malaria transmission in these areas. Other Anopheles species such as An. gambiae, which breed in open sunlit stagnant water pools [9, 20, 34] are also highly anthropophagic and generally occur near human habitations [35]. The ability of An. funestus to breed in the river waters is not unique to Tanzania, but has also been demonstrated in other places such as coastal Kenya [9, 21], and may be due to the higher levels of aeration and dissolved oxygen in such waters. Additional investigations are therefore required to further examine these details. A similar ecological niche has been described for An. pseudopunctipennis in South America, which was successfully controlled by clearing the river waters of the algal blooms [36].

While it is unclear whether clearing the identified habitats of emergent vegetation would be suitable for control of An. funestus in Tanzania, it will be important to investigate it as a potential environmentally-friendly approach, in which community members could be engaged to achieve effective disease prevention. Besides, it will be important to ascertain the importance of these habitat types across multiple sites and settings. For instance, in one area in the north of Tanzania, Dida et al., [37] found no mosquito larvae near the main rivers, suggesting the dominant malaria vectors may be breeding elsewhere in such settings.

Understanding the physicochemical characteristics of mosquito larval habitats is also important in understanding their overall ecological needs, and assessing options for manipulation. In this study, it is possible that the physicochemical parameters might have been influenced by agricultural practices and pesticide use, which are prevalent in the valley (Matowo et al., unpublished). Emerged adult mosquitoes from these aquatic habitats might become more resistant towards the insecticides having the same chemical formula used in mosquito vector control [35, 38, 39]. Here we found that the most prolific habitats were located at higher altitudes, perhaps less affected by agricultural insecticidal deposits [40, 41], than the floor of the valley. Nonetheless, the mosquito species from the same study villages are known to be already strongly resistant to insecticides used for public health, including pyrethroids and carbamates (Pinda et al., unpublished), a situation potentially related to widespread use of pesticides in both agriculture and public health. Similar to other studies on Anopheles mosquitoes, the An. funestus habitats in this study area had weak acidity pH [39, 42]. The main habitats had pH ranging from 6.5 to 6.7, turbidity from 26.6 to 54.8 NTU and total dissolved solids from 60.5 to 80.3 mg/L, all of which are similar to most observations of habitats of Anopheles mosquitoes in previous studies [43, 44]. Some of An. funestus mosquitoes were collected from the habitats with moderate concentration of nitrate, indicating the possibility that they use it as a source of nutrients during larval development as earlier described [39].

Overall, this study has provided the basic description of An. funestus habitats in rural south-eastern Tanzanian districts of Ulanga and Kilombero. The effective control measures for this species should consider understanding their behavior and ecology including characteristics of their aquatic habitats so that can be targeted during their immature stages. Given the rarity of the An. funestus habitats and the observed characteristics, these habitats fit the description of being fixed, few and findable. Future studies should therefore investigate potential of using larviciding or larval source management to improve malaria control in settings where An. funestus dominate.

# Conclusion

Improved understanding of the larval ecology of An. funestus is an essential step in achieving the targets of current and future malaria control strategies. This study has identified and described major aquatic habitats of the dominant malaria vector, An. funestus in rural south-eastern Tanzania, and will form an important basis for further ecological studies and improved control of this mosquito species. The study examined more than 100 potential habitats across six villages, and identified three main habitat types as follows: a) small water well, which have well-defined edges, are occasionally shaded by trees, are spring-fed and are occupied by multiple species of the An. funestus group, b) medium-sized ponds, for which the central part retains water for all or most of the year, have surface vegetation and are occupied by multiple other Anopheles species such as An. arabiensis, and c) riverine habitats consisting of the slow-moving waters on rivers or river tributaries, also with vegetation. These habitats were mostly found at altitudes above 400 m above sea level unlike the other two habitats, which were more common at lower altitudes below 300 m. In summary, An. funestus in rural south-eastern Tanzania appear to prefer permanent and semi-permanent aquatic habitats characterized by stagnant or slow-moving waters, emergent vegetation e.g. algae on swamp surfaces, clear waters at depths exceeding 50 cm, and nearness to human dwellings. Given the observed characteristics, An. funestus habitats in the area can indeed be described as fixed, few and findable. Future studies should therefore investigate potential of targeting these habitats with larviciding or larval source management to complement malaria control efforts in areas dominated by this vector.

# Abbreviations

WHO: World Health Organization; PCR: Polymerase chain reaction; NTU: Nephelometric turbidity unit; LLINs: Long lasting insecticide-treated nets; IRS: Indoor Residual Spraying; LC: Lower class limit; UC: Upper class limit.

# Declarations

## Author contributions

IHN and FOO conceived the study, developed the research idea and study protocol and produced initial and final drafts of the manuscript. IHN, HSN and FOO analyzed the data. EEH and SVM assisted in molecular identification of species and physicochemical analysis respectively. IHN, DSM and BJM produced the study site maps. IHN, NTM, KRM and GZT identified and characterized the larval habitats. IHN, HSN, EWK, MFF, BJM, RMN, SAM, DMM, EEH, DWL, NJG and FOO contributed to drafting the manuscript. All authors have read and contributed to the final manuscript.


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## Conflict of interest

The authors declared that, they have no competing interest.

## Availability of data and materials

All data generated from this study will be available from the corresponding author as per request.

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**Ethical statement**

Ethical approval for this study was obtained from Ifakara Health Institute Institutional Review Board (IHI/IRB/No:19-2017), and from National Institute of Medical Research (NIMR) through Medical Research Coordinating Committee (MRCC), Ref: NIMR/HQ/R.8c/Vol. I/1185. Meetings were held with community leaders of each village, where the purpose and procedures of the study were explained, and permission was sought from the communities to inspect the mosquito larval habitats and sample larvae in their respective villages.

**Consent for publication**

This manuscript has been approved for publication by the National Institute of Medical Research, Tanzania (NIMR/HQ/P.12 VOL XXX/15).

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# Tables

**Table 1:** Characteristics of aquatic habitats of *An. funestus* and other mosquito species

| Larval habitat | All water bodies n (%) | Water bodies without larvae n (%) | Habitats with *An. funestus* n (%) | Habitats with other *Anopheles* n (%) | Habitats with culicines n (%) |
|---|---|---|---|---|---|
| **Water movement** | | | | | |
| Stagnant | 91 (82) | 25 (89.3) | 27 (75) | 18 (75) | 21 (91.4) |
| Slow | 20 (18) | 3 (10.7) | 9 (25) | 6 (25) | 2 (4.3) |
| **Habitat shade** | | | | | |
| None | 60 (54.1) | 11 (39.3) | 17 (47.2) | 18 (75) | 14 (60.9) |
| Partial | 35 (31.5) | 14 (50) | 11 (30.6) | 4 (16.7) | 6 (26.1) |
| Heavy | 16 (14.4) | 3 (10.7) | 8 (22.2) | 2 (8.3) | 3 (13) |
| **Water depth** | | | | | |
| Less than 50cm | 51 (45.9) | 12 (42.9) | 12 (33.3) | 17 (70.8) | 10 (43.4) |
| Greater than 50cm | 60 (54.1) | 16 (57.1) | 24 (66.7) | 7 (29.2) | 13 (56.6) |
| **Distance to human dwellings** | | | | | |
| Less than 100m | 77 (69.4) | 26 (92.9) | 28 (77.8) | 9 (37.5) | 14 (60.9) |
| Greater than 100m | 34 (3.6) | 2 (7.1) | 8 (22.2) | 15 (62.5) | 9 (39.1) |
| **Water type** | | | | | |
| Permanent | 44 (39.6) | 7 (25) | 21 (58.3) | 7 (29.2) | 9 (39.1) |
| Semi-permanent | 67 (60.4) | 21 (75) | 15 (41.7) | 17 (70.8) | 14 (60.9) |
| **Vegetation type** | | | | | |
| Emergent | 51 (46) | 6 (21.4) | 26 (72.2) | 7 (29.2) | 12 (52.2) |
| Submerged | 11 (9.9) | 7 (25) | 1 (2.8) | 1 (4.2) | 2 (8.7) |
| None | 25 (22.5) | 12 (42.9) | 3 (8.3) | 6 (25) | 4 (17.4) |
| Floating | 24 (21.6) | 3 (10.7) | 6 (16.7) | 10 (41.6) | 5 (21.7) |

**Table 2:** Results of univariate and multivariate regression analysis of different habitat characteristics and their association with presence of *An. funestus* larvae

| | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| **Larval habitat** | **Odds (95% LC, UC)** | **P- values** | **Odds (95% LC, UC)** | **P- values** |
| **Water movement** | | | | |
| Stagnant | 1 | | 1 | |
| Slow | 1.94 [0.72, 5.21] | 0.189 | 3.71 [0.81, 17.00] | 0.091 |
| **Habitat shading** | | | | |
| None | 1 | | 1 | |
| Partial | 1.16 [0.47, 2.87] | 0.750 | 0.84 [0.22, 3.24] | 0.795 |
| Heavy | 2.53 [0.82, 7.83] | 0.107 | 7.35 [1.04, 51.78] | <0.05 |
| **Water depth** | | | | |
| Less than 50 cm | 1 | | 1 | |
| Greater than 50 cm | 2.17 [0.95, 4.96] | 0.067 | 5.72 [1.40, 23.42] | <0.05 |
| **Distance to human dwellings** | | | | |
| Less than 100m | 1 | | 1 | |
| Greater than 100m | 0.54 [0.21, 1.35] | 0.186 | 0.43 [0.12, 1.49] | 0.184 |
| **Water type** | | | | |
| Semi-permanent | 1 | | 1 | |
| Permanent | 3.16 [1.39, 7.23] | <0.01 | 3.07 [0.86, 10.99] | 0.085 |
| **Vegetation type** | | | | |
| None | 1 | | 1 | |
| Submerged | 0.73 [0.07, 7.95] | 0.799 | 0.55 [0.21, 1.42] | 0.216 |
| Emergent | 7.63 [2.03, 28.70] | <0.01 | 1.96 [0.66, 5.78] | 0.966 |
| Floating | 2.44 [0.53, 11.17] | 0.249 | 0.92 [0.03, 31.86] | 0.962 |

**Table 3:** Median values of key physicochemical parameters in aquatic habitats dominated by different mosquito species

| Dominant mosquito species | pH | Turbidity (NTU) | Temp (ºC) | Conductivity (µS/cm) | TDS (mg/L) | Nitrate (mg/L) |
|---|---|---|---|---|---|---|
| ***Anopheles funestus*** | 6.6<br>(6.5-6.7) | 32.9<br>(26.6-54.8) | 27.1<br>(25.2-28.8) | 151.0<br>(134-165) | 69.7<br>(60.5-80.3) | 4.1<br>(2.9-6.6) |
| **Culicine mosquitoes** | 6.2<br>(5.9-6.5) | 36.0<br>(19.8-43.2) | 26.5<br>(25.1-27.3) | 61.0<br>(106-189) | 78.8<br>(53.1-112.0) | 2.7<br>(1.6-3.7) |
| **Other *Anopheles*** | 6.8<br>(6.4-7.1) | 24.9<br>(19.4-64.0) | 28.9<br>(23.2-32.6) | 211.0<br>(123-251) | 102.0<br>(50.3-108.0) | 10.3<br>(2.4-45.5) |
| **Without larvae** | 6.41<br>(5.8-6.7) | 15.6<br>(11.5-20.2) | 25.6<br>(24.5-27.0) | 286.0<br>(99.2-310.0) | 142.0<br>(88.7-148.0) | 2.45<br>(1.4-2.9) |

**Table 4:** Univariate and multivariate analysis of associations between physicochemical parameters and the presence of *An. funestus* larvae

| | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| **Characteristics** | **Odds (95% LC, UC)** | **P- values** | **Odds (95% LC, UC)** | **P value** |
| pH | 1.40 [0.57, 3.44] | 0.458 | 3.77 [0.96, 14.84] | 0.057 |
| Temperature | 0.99 [0.86, 1.14] | 0.896 | 0.92 [0.76, 1.12] | 0.403 |
| Nitrate | 0.98 [0.94, 1.02] | 0.368 | 0.94 [0.83, 1.01] | 0.073 |
| TDS | 0.99 [0.98, 1.00] | 0.197 | 0.98 [0.94, 1.02] | 0.248 |
| Turbidity | 1.01 [0.99, 1.01] | 0.285 | 1.01 [0.99, 1.02] | 0.231 |
| Conductivity | 1.00 [0.99, 1.00] | 0.272 | 1.01 [0.99, 1.02] | 0.507 |

# Figures

## Figure 1

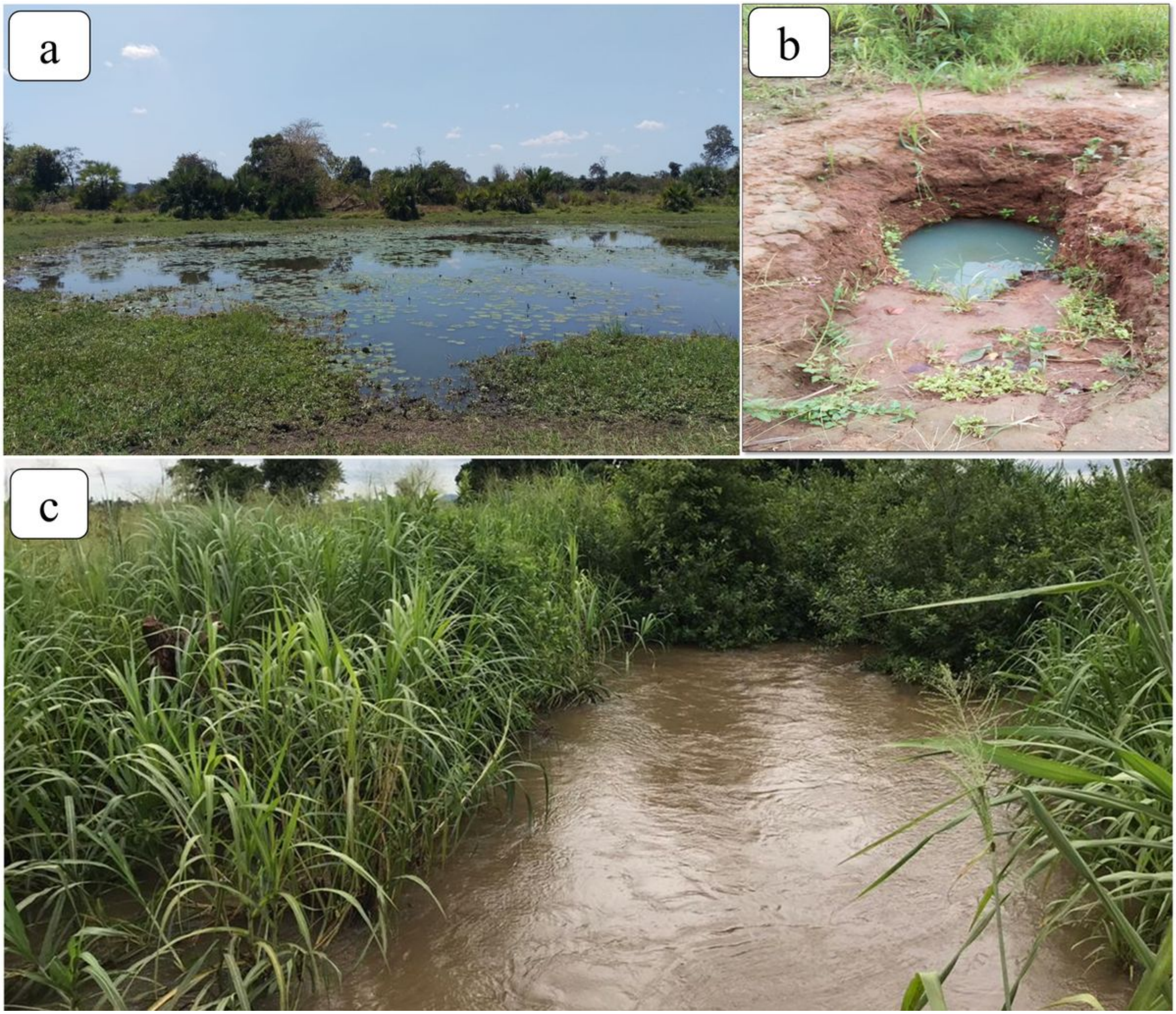
Map of Kilombero and Ulanga districts showing the six study villages.

## Figure 2

Collection of An. funestus larvae using 10 L bucket (a) and standard dipper (b).

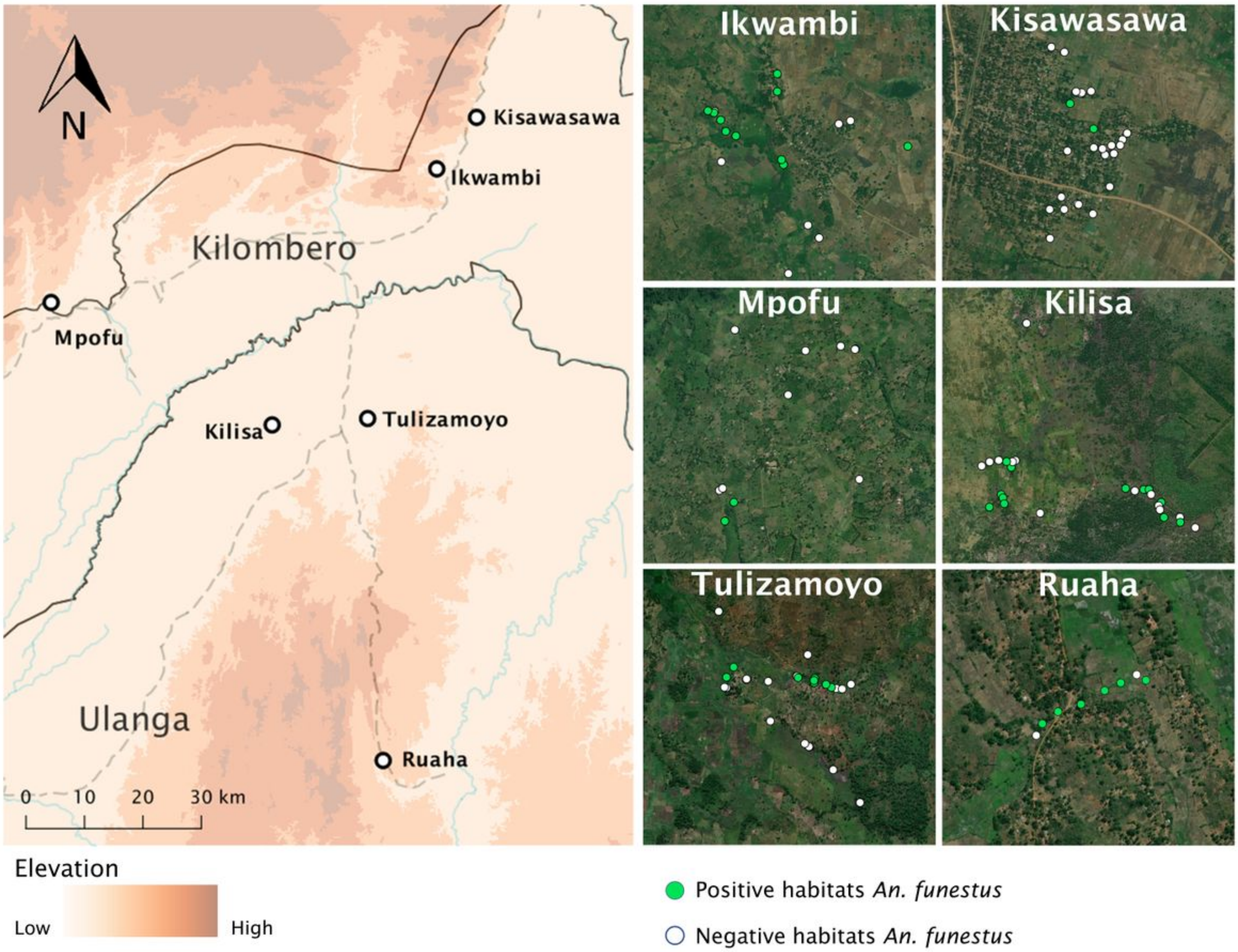
## Figure 3

Picture of a riverside aquatic habitat for Anopheles funestus mosquitoes, as identified in the study areas in rural south-eastern Tanzania. In altitudes above 400 m, these were the only An. funestus habitats identified.




## Figure 4

Typical larval habitats of Anopheles funestus mosquitoes in lower altitude areas (a; medium-sized ponds that retain water at the center most of the year and have emergent surface vegetation & b; small spring-fed wells with well-defined perimeters) and habitats at higher altitudes (c; slow-moving waters at the river side with emergent vegetation).




## Figure 5

Spatial distribution of Anopheles funestus larval habitats in the selected study villages in south-eastern Tanzania.