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Spatial and temporal distribution of *Anopheles* mosquito's larvae and its determinants in two urban sites in Tanzania with different malaria transmission levels

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ABSTRACT

Background: In order to be able to design and implement control measures directed to the mosquito larva stages an understanding of the spatial and temporal distribution and its determinants in different malaria transmission settings is important. This study therefore, intended to determine the spatial and temporal distribution of *Anopheles* mosquito's larvae and its determinants in two urban sites with different transmission levels, in Tanzania.

Methodology: This study was conducted in Dodoma and Morogoro regions in Tanzania. The study was an ecological study of repeated cross-sectional type. Searching for water bodies in the selected wards was done by going around all streets. Potential breeding sites were given unique identification numbers and larval sampling was done using the standard dipping method with a 350 ml mosquito scoop and a calibrated pipette. Visual identification of presence of larvae and its abundance in each sampling were used to describe the larvae density. A sample of *Anopheles* mosquitoes which emerged from collected larvae, were processed for species identification using PCR. Descriptive statistics were arrived at by calculating different proportions for the variables. The overall impact of the variables on the density of *Anopheles* larvae was tested using multiple logistic regression. Variables with *p*-value less than 0.05 were regarded as significant.

Results: A total of 724 water bodies out of which, 576 (79.6%) potential breeding sites were analyzed. It was found that, most (96.2%) of the potential breeding sites were manmade and most (59.5%) were less than 5 m in diameter and 87.2% were within 100 m from human settlement. Out of all the potential breeding sites, 69.8% and 30.2% were in Morogoro and Dodoma respectively, out of which 72.2% and 68.4% respectively, were found during rainy season. Habitats with clean water, at a distance of 10–100 m from the house, in natural, shaded and partial sunlight habitats had higher odds of having high density of mosquito larvae than their counterparts ($p < .05$). The PCR analysis showed that 72.5% were *An. arabiensis,* 4.5% *An. gambiaes.s,* 0.5% *An.coustani*and 20% *An. quadrianulatus*while 2.5% of the samples could not be identified because DNA was not amplified.

Conclusion and recommendation: Type of water, distance from the breeding site to human settlement, light intensity and habitat origin were significant predictors of variation on the spatial and temporal distribution of Anopheles mosquito breeding sites. With increased global emphasis on control measures that targets mosquito immature stages; we recommend that larval control measures should be developed while considering the findings from this study.

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1. Background

Malaria is a disease of great public health significance in many developing countries in the tropics, mostly Africa south of Sahara (Gillies and Coetzee, 1987). The disease is caused by a blood dwelling protozoan parasites of the genus Plasmodium, which is almost exclusively transmitted through the bite of infected female anopheles mosquitoes (WHO, 2012; Kelly-Hope et al., 2009; Kebede et al., 2017).The vector Mosquitoes lays eggs in a wide range of aquatic niches whose distribution determines mosquito abundance and consequently malaria transmission risk (Herrera-Varela et al., 2014).

In 2019 an estimate of 228 million cases of malaria occurred worldwide, with nineteen (19) countries in sub-Saharan Africa and India carrying almost 85% of the global malaria burden and close to 94% of all malaria deaths in the world for which about 67% occurs among children under the age of five (WHO, 2020). Significant strides have been made in malaria prevention and control for the past decades in Tanzania, however, the disease remains a significant public health concern with about 93% of the population at risk of infection and it remains the leading ten years cause of inpatient hospital deaths in the country (WHO, 2018; Mboera et al., 2018).

Vector control has been a fundamental tool in any sustainable vector-borne disease control programme (Scott and Morrison, 2010). The use of Long Lasting Insecticide Nets (LLIN) and Indoor Residual Spraying (IRS) have been the current most effective Malaria vector control tools (Beier et al., 2008). Pyrethroids are the only insecticides currently available for use on bednets. It is clear that, because resistance to these compounds is widespread in Africa, these methods are progressively becoming less effective (Yawson et al., 2004; Diabaté et al., 2002; Chandre et al., 1999), thus, advances in vector control by identifying and targeting on the most productive larval habitat and breeding sites are very essential (Elimam et al., 2009). Owing to the limited movement of mosquito immature stages compared with that of free-flying adult mosquitoes, control of the immature stages is more efficient and necessary (Blaustein and Kotler, 1993). In order to be able to design and implement control measures directed to the larva stages such as larva source reduction and larviciding, an understanding of the spatial and temporal distribution of malaria mosquito larva and its determinants in different malaria transmission settings is important (Maheu-Giroux and Castro, 2014; Maheu-Giroux and Castro, 2013). This study therefore, intended to determine the spatial and temporal distribution of *Anopheles* mosquito's larvae and its determinants in two urban sites with different malaria transmission rates, in Tanzania.

2. Methodology

2.1. Study area

This study was conducted in two regions which were Dodoma and Morogoro in Tanzania. Morogoro and Dodoma regions were conveniently selected to represent high and low malaria prevalence areas with permanent and seasonal mosquito breeding sites respectively. Morogoro is among regions with high prevalence of malaria (9.5%) whereas Dodoma falls into regions with low malaria prevalence (0.6%) (NBS, 2017).

Morogoro municipality which has a population of 350,000 (NBS, 2013) is found in the Eastern part of Tanzania, 169 km west of Dar-es-Salaam the country's largest city and commercial centre and 223 km east of Dodoma the country's capital city. Morogoro town (6°49′S and 37°40′E) lies at the foot of the Uluguru Mountains at an average altitude of 522 m above mean sea level. It experiences short rains during December and January and long rains from March to June. Total average annual rainfall is 783.5 mm, mean relative humidity 72%, minimum temperature 22 °C, and maximum temperature 33 °C during wet seasons (December–May). During cold season (June–September) minimum and maximum temperatures are 15 °C and 19 °C respectively.

Dodoma municipality which is located at 6°25′S and 35°75′E is 486 km west of Dar-es-Salaam and has a population of 410,956 (NBS, 2013). It covers an area of 2669 km^2 of which 625 km^2 are urbanized. It is semi-arid, with total annual average rainfall 478.4 mm, mean relative humidity 67%. During the wet season minimum and maximum temperatures are 22 °C, and 31 °C respectively while in the cold season (June–September) the minimum and maximum temperature are to 13 °C 18 °C. The town receives short rains in December and long rains from February to March.

2.2. Study design

The study was an ecological study of repeated cross-sectional type. This survey was conducted in two regions, Dodoma and Morogoro as described in study area section. The data were collected in two seasons, cold-dry season from June to September 2014 and in hot-wet seasons from January to February 2015. In all two seasons larvae mosquitoes were collected in same locations. Larvae were sampled from all encountered potential breeding sites during the study period.

2.3. Sampling techniques

Purposively, two wards were selected in Dodoma and Morogoro urban based on presence of seasonal and temporary breeding sites (presence of artificial breeding sites, natural swamps and rice paddies) with local high incidence of malaria within the wards (MTUHA, 2020a; MTUHA, 2020b).

2.4. Data collection procedure

2.4.1. Breeding site identification

Searching for water bodies in the areas under study was done by going around all streets of the wards. Ten-Cell leaders of these streets in the wards lead the research team into different places within the wards for identification and location of water bodies which may contain *Anopheles* mosquitoes. Once the water bodies were found to have *Anopheles* larvae, researchers recorded that water body as potential breeding site. Each potential breeding site encountered in the study area was given a unique identification number and its position was recorded using hand-held Global Positioning System (GPS) device. Thereafter, classification of potential breeding sites in different habitat characteristics was done using visual observation and tape measure. The parameters such as water depth, habitat size, distance of water body to houses, water type, water current and surrounding land cover was recorded for every accessible potential aquatic mosquito habitat during study survey. Water depth was classified as shallow when the water level was <0.5 m (e.g. hoof prints, burrow pits, tyre tracks) and deep when it was >0.5 m (e.g. ditches, holes). Habitats that had diameter of one meter or less were categorized as small breeding sites (e.g. water in containers, rain pools) whereas for those with diameter greater than one meter were categorized as large breeding habitats (e.g. rice paddies) (Sattler et al., 2005).

Distance of the potential habitat to the human dwelling was measured by tape measure and three categories were recorded, <10 m, 10–100 m and >100 m. Water types (clean or polluted), light intensity (full sunlight, partial sunlight and shaded) and water current (stagnant or slow flow) were recorded using visual perceptions. All visual classifications were done by the same person to maintain consistency. Lastly, the surrounding land cover around each potential aquatic habitat was recorded as long grasses, short grasses and type of vegetation e.g. rice. All these parameters were recorded on larval collection form.

2.4.2. Larval collection

Sampling of *Anopheles* larvae were conducted in the morning around 0700 h to 1100 h during the study periods (June to September 2014 and January to February 2015). Larvae sampling was done using the standard dipping method with a 350 ml mosquito scoop as described by Service (Service MW, 2000). For small water bodies where a scoop could not be applied, larva was collected using calibrated pipette. Visual identification of presence of larvae and its abundance in each sampling were used to describe the larvae density. When larvae were seen without sampling or when nearly every sampling contained *Anopheles* species that habitat was recorded as having high *Anopheles* larvae density and if sampling contained few *Anopheles* larvae the habitat was categorized as having low *Anopheles* larvae density. After collection, mosquito larvae were taken to the laboratory for *Anopheles* species identification. Positioning of the larvae parallel to the water surface and absence of siphon were used to identify *Anopheles* larvae. Pupae were excluded from the study as they could not be visually identified from the field. Thereafter larvae were taken to the insectary (with temperature of 28 ± 5 °C, relative humidity of 70–78%) and reared until they emerged to adult mosquitoes so that they could be identified by amplification of ribosomal DNA using polymerase chain reaction (PCR) (Scott et al., 1993).

2.4.3. Dissection of mosquito and DNA extraction

A sample of *Anopheles* mosquitoes which emerged from collected *Anopheles* larvae, were processed for species identification using PCR. DNA of each of the sampled *Anopheles* mosquito was extracted for PCR amplification. A 100 μl Bender Buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA pH 9.1, and 0.5% SDS in DEPC water) was used to homogenize the head, thorax and legs until there were no recognizable mosquito parts. Eight moles of potassium acetate were added to the homogenized samples and incubated for one hour then spun. Thereafter 300 μl 100% ethanol was added to supernatant to precipitate DNA, then centrifuged to obtain small pellet of DNA. This DNA pellet was stored at −20 °C until used for PCR analysis.

2.4.4. Polymerase chain reaction (PCR) analysis for differentiation of Anopheles species

The differentiation of the *Anopheles gambiae* complex by PCR was done by using PCR protocols by Scott et al. (Scott et al., 1993). The PCR amplification was performed with universal and species specific primers for the *An. gambiae* complex. Molecular identification of *An. gambiae* complex was based on three deferentially sized amplicons of the species-specific nucleotide sequences in the ribosomal DNA intergenic spacer (IGS). The extracted product sizes were as follows: *An. gambiae*s.s. (~390 bp), *An. arabiensis* (~315 bp), and *An. quadriannulaus*(~150 bp) (Scott et al., 1993). The amplified DNA was separated on a 2.0% agarose gel stained with ethidium bromide and viewed on a UV transilluminator. The following were the primers for *An. gambiae* complex:

Primers:

UN: 5′- GTG TGC CCC TTC CTC GAT GT -3′
GA: 5′- CTG GTT TGG TCG GCA CGT TT -3′
AR: 5′- AAG TGT CCT TCT CCA TCC TA -3′
QD: 5′- CAG ACC AAG ATG GTT AGT AT -3′

2.5. Data processing and analysis

Data on different habitat characteristics, presence and density of mosquito larvae were entered into SPSS V.16. Descriptive statistics were arrived at by calculating different proportions for the variables. The impact of different water body characteristics on the density of mosquito larvae was explored individually. Comparisons between proportions were made using Chi-square test. All variables were incorporated in a mathematical model and their overall impact on the density of *Anopheles* larvae tested using multiple logistic regression. Variables with *p*-value less than 0.05 in multiple logistic regression models were retained for interpretation.

2.6. Ethical considerations

Ethical clearance for the studies was granted by the Sokoine University of Agriculture (SUA) Directorate for Research and Postgraduate studies. Permission to conduct the study was sought from the district and respective ward authorities.

3. Results

3.1. Characteristics of the surveyed aquatic habitats

Table 1 shows the aquatic characteristics of water bodies surveyed in the study areas. A total of 724 water bodies were analyzed in two study areas during dry and rainy seasons. Out of these, 576 (79.6%) which were potential breeding sites for mosquito larvae were analyzed. Among the surveyed potential breeding sites, 69.8% were found in Morogoro of which 72.2% were found during rainy season. In Dodoma positive aquatic habitat for *Anopheles* larvae were reported to be 30.2% and majority (68.4%) of those habitats were visited during rainy season. On the occasions when study were conducted, it was observed that 96.2% of potential breeding habitats were manmade in origin (e.g. brick pits) while only 3.8% were naturally occurring (e.g. swamps). Interestingly, the study found out that, 18.8% water bodies that were positive for *Anopheles* larvae were polluted with either human or animal excreta. Moreover, 38.9% of potential polluted breeding sites were observed during the rainy season (Table 1).

3.2. Characteristics of potential breeding sites for Anopheles in the study sites

Table 2 shows the characteristic of potential breeding sites with respect to presence of *Anopheles* larvae in the two study sites. The results show that *Anopheles* larvae were spatially distributed in different aquatic habitats. Out of the 576 sampled habitats that had *Anopheles* larvae, 25.2% were rice paddies, 23.3% were ditches, 21.8% were containers and 18.8% were septic tanks/pits. Furthermore, 59.5% of the habitats had size less than five meters diameter while 24% were of diameter 5–10 m and 16.5% had diameter greater than 10 m. It was further observed that half (50%) of the breeding sites were within 10–100 m from human settlements, while 37.2% and 12.8% were within less than 10 m and more than 100 m respectively from the human settlement. Slightly more than half (51.9%) of the breeding sites were covered by short and long grasses while 40.5% were covered by short grass. It was observed that 34.2% of the breeding sites were shaded while 45.8% had partial sunlight and 20% had full sunlight. It was observed that all (100%) of the aquatic habitats which contained *Anopheles* larvae were stagnant water. Additionally, during the survey it was found that in 33.9% of the breeding sites, *Anopheles* and Culicine species occurred concurrently (Table 2).

The findings further shows that majority 119 (68.4%) and 290 (72.1%) of breeding sites in Dodoma and Morogoro respectively were visited during wet seasons, of which rice paddies were the major (33.4%) breeding sites encountered in Morogoro and containers (40.3%) in Dodoma. During dry seasons most of breeding sites were dried off thus the main breeding habitats in Morogoro were ditches (41.1%) and septic tanks (35.7%) while in Dodoma were containers (40.3%) and septic tanks (40.0%). Furthermore, it was also observed that during dry and wet seasons majority of surveyed breeding site had size of ≤5 m, and most of them 42.5%

Table 1
Aquatic habitat characteristics of surveyed potential mosquito breeding sites in Dodoma and Morogoro according to seasons (n = 576).

Variable	Total n (%)	Rainy season n (%)	Dry seasonn (%)
Surveyed Potential breeding sites			
Dodoma	174 (30.2)	119 (68.4)	55 (31.6)
Morogoro	402 (69.8)	290 (72.2)	112 (27.8)
Origin of habitat			
Manmade	554 (96.2)	389 (70.2)	165 (29.8)
Natural	22 (3.8)	20 (90.9)	2 (9.1)
Type of water bodies			
Clean	468 (81.2)	367 (78.4)	101 (21.6)
Polluted	108 (18.8)	42 (38.9)	66 (61.1)

Table 2
Characteristics of potential breeding sites with respect to sampling frequency of *Anopheles* mosquitoes collected in Dodoma (n = 174) and Morogoro (n = 402).

Variables		Dodoma			Morogoro		
	Total n (%) N = 576	% Total (n = 174)	% Rain season (n = 119)	% Dry season (n = 55)	% Total (n = 402)	% Rain season (n = 290)	% Dry season (n = 112)
Habitat types							
Brick pits	18 (3.1)	8 (4.6)	8 (6.7)	0(0.0)	10 (2.5)	10 (3.4)	0(0.0)
Containers	126 (21.8)	49 (28.2)	48 (40.3)	21 (38.2)	77 (19.2)	51 (17.6)	26 (23.2)
Ditches	134 (23.3)	31 (17.8)	19 (16.0)	12 (21.8)	97 (24.1)	57 (19.7)	46 (41.1)
Foot print	20 (3.6)	6 (3.5)	6 (5.0)	0 (0.0)	14 (3.5)	14 (4.8)	0(0.0)
Rice paddies	145 (25.2)	48 (27.6)	28 (23.5)	0 (0.0)	102 (25.6)	97 (33.4)	0(0.0)
Septic tanks/pits	108 (18.8)	32 (18.4)	10 (8.5)	22 (40.0)	76 (18.9)	36 (12.5)	40 (35.7)
Swamps	21 (3.6)	0(0.0)	0(0.0)	0(0.0)	21 (5.2)	21 (7.3)	0(0.0)
Tyre tracks	4 (0.6)	0(0.0)	0(0.0)	0(0.0)	4 (1.0)	4 (1.3)	0(0.0)
Size of habitat (m)							
≤ 5	343 (59.5)	82 (47.1)	63 (52.9)	31 (56.4)	261 (64.9)	151 (52.1)	110 (98.2)
5–10	138 (24.0)	44 (25.3)	32 (26.8)	24 (43.6)	94 (23.4)	94 (32.4)	0(0.0)
≥10	95 (16.5)	48 (27.6)	24 (20.3)	0(0.0)	47 (11.7)	45 (15.5)	2 (1.8)
Distance from house to breeding site (m)							
≤10	214 (37.2)	58 (33.3)	45 (37.8)	18 (32.7)	140 (34.8)	80 (27.5)	50 (44.6)
10–100	288 (50.0)	74 (42.5)	40 (33.6)	29 (52.7)	230 (57.2)	180 (62.1)	60 (53.6)
≥100	74 (12.8)	42 (24.2)	34 (28.6)	8 (14. 6)	32 (8.0)	30 (10.4)	2 (1.8)
Light intensity							
Shaded	197 (34.2)	45 (25.9)	38 (31.9)	7 (12.7)	152 (37.8)	109 (37.6)	43 (38.4)
Partial Sunlight	264 (45.8)	76 (43.6)	60 (50.4)	32 (58.2)	211 (52.5)	181 (62.4)	30 (26.8)
Full Sunlight	115 (20)	53 (30.5)	21 (17.6)	16 (29.1)	39 (9.7)	0(0.0)	39 (34.8)
Land Cover							
Short grasses (≤30 cm)	233 (40.5)	74 (42.5)	33 (27.7)	41 (74.5)	159 (39.6)	91 (31.4)	68 (60.7)
Combination (Short and long grasses)	299 (51.9)	78 (44.8)	68 (57.2)	10 (18.2)	221 (54.9)	178 (61.4)	43 (38.4)
Under tree	44 (7.6)	22 (12.6)	18 (15.1)	4 (7.3)	22 (5.5)	79 (27.2)	1 (0.9)
Water Current							
Running (slow)	233 (40.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stagnant	299 (51.9)	174 (100)	119 (100)	55 (100)	402 (100)	290 (100)	112 (100)
Coexistence of mosquito species							

(continued on next page)

in Dodoma and 57.2% in Morogoro had a distance of 10–100 m from human settlement. With respect to co-existence of *Anopheles* with other species, it was revealed that during wet season Dodoma and Morogoro did not differ much in terms of breeding sites either *Anopheles* alone or a mixture of *Anopheles* and Culicines. While in dry season most (68.7%) breeding sites in Morogoro displayed coexistence of *Anopheles* and Culicnes in compared with Dodoma where most (60%) of the breeding sites had *Anopheles* larvae alone (Table 2).

3.3. Anopheles larval density by habitat characteristic

The results of univariate analysis using Chi-square test showed that the *Anopheles* larval density was significantly associated with season (p = .017), type of water (p = .020), light intensity (p = .001), and habitat origin (p = .001). It was noted that, 59.4% and 70.1% of the aquatic habitats in rain and dry season respectively, had high *Anopheles* larvae density (p = .017). *Anopheles larvae* were found in 59.0% and 68.7% of clean and polluted water habitats respectively (p = .02). This study, further observed that, 70.1% and 58.9% of the habitats in partial and in shaded sunlight respectively, had high mosquito larval density, while only 51.3% of the habitats in full sunlight had high mosquito larval density (p = .001). It was further noted that, majority of the manmade larval habitats (63.9%) had high larval density as compared to natural habitats (27.3%), (p = .001). With regards to the breeding sites surrounded by grasses, 67.8% of habitants with short grasses had high *Anopheles* larval density compared 58.9% of those with combination of short and long grass, and 59.1% of those under tree (p = .09) (Table 3).

3.4. Factors associated with Anopheles larval density

The analysis of multiple logistic regression model adjusting for season, type of water, size of habitats, distance from house to breeding site, light intensity, land cover, habitants origin, surveyed sites and water current demonstrated that the effect of season on larval density was no longer significant (p = .21). Water current (p = .9365), land cover (p = .6162), size of habitat (p = .6428), and surveyed site (p = .1958) were also not significantly associated with larval density so were removed from the final model. The adjusted odds ratios of the final fitted model for the risk factors associated with larval density are presented

Table 3
The distribution of larval density according to breeding sites characteristics.

Variable	High density n (%)	Low density n (%)	p-value
Season			
Rain	243 (59.4)	166 (40.6)	.017
Dry	117 (70.1)	50 (29.9)	
Type of water			
Clean	217 (59.0)	151 (41.0)	.020
Polluted	143 (68.7)	65 (31.3)	
Distance from house to breeding site (m)			
10	184 (63.9)	104 (36.1)	
10–100	126 (58.9)	88 (41.1)	.3254
>100	50 (67.6)	24 (32.4)	
Light intensity			
Shaded	116 (58.9)	81 (41.1)	
Partial sunlight	185 (70.1)	79 (29.9)	.001
Full sunlight	59 (51.3)	56 (48.7)	
Land Cover			
Combination (Short and long grasses)	176(58.9)	123(41.1)	.0949
Under tree	26 (59.1)	18 (40.9)	
Short grasses (≤30 cm)	158 (67.8)	75 (32.2)	
Habitat Origin			
Manmade	354 (63.9)	200 (36.1)	.001
Natural	6 (27.3)	16 (72.7)	
Surveyed sites			
Dodoma	101 (58.0)	73 (42.0)	.146
Morogoro	259 (64.4)	143 (35.6)	

Table 4
Multiple Logistic regression for factor associated with larval density.

Variable	AOR	95% CI	p-value
Type of water			
Clean	1.50	[1.031, 2.185]	.0340
Polluted	Reference		
Distance from house to breeding site (m)			
<10	1.20	[0.685, 2.114]	.5185
10–100	1.90	[1.064, 3.405]	.0300
>100	Reference		
Light intensity			
Shaded	2.16	[1.348, 3.468]	.0014
Partial Sunlight	1.78	[1.193, 2.650]	.0047
Full Sunlight	Reference		
Habitat Origin			
Manmade	Reference		
Natural	4.09	[1.501, 11.156]	.0059

Table 5
Species composition of emerged *Anopheles* mosquitoes collected from larvae collection in study sites.

Species	Dry season n (%)	Wet season n (%)	Total n (%)
Anopheles arabiensis	96 (96.0)	49 (49.0)	145 (72.5)
Anopheles gambiaes.s	2 (02.0)	7 (07.0)	9 (04.5)
An.coustani	0(00.0)	1 (01.0)	1 (00.5)
An. quadrianulatus	0 (00.0)	40 (40.0)	40 (20.0)
Not amplified	2 (02.0)	3 (03.0)	5 (02.5)
Total	100 (100.0)	100 (100.0)	200 (100.0)

in Table 4. Aquatic habitats with clean water were significantly more likely to have high density of *Anopheles* mosquito larvae than those with polluted water ($p = .0340$). Habitats at a distance of 10–100 m from the house had almost two times higher odds of having high density of mosquito larvae than habitats more than 100 m from a house ($p = .03$). With respect to light intensity, habitats in shaded ($p = .0014$) and partial sunlight ($p = .0047$) were significantly more likely to have high density of mosquito larvae than habitants in full sunlight. The odds of having high density of mosquito larvae for natural habitats was 4.09 times that of manmade habitats ($p = .0059$) (Table 5).

3.5. Molecular identification

3.5.1. Polymerase chain reaction (PCR) analysis

Out of 1426 *Anopheles* mosquitoes which emerged from collected *Anopheles* larvae, a sample of 200 *Anopheles* mosquitoes (100 from dry season and 100 rainy season) were randomly selected and then processed for species identification using PCR. The PCR analysis showed that 72.5% were *An. arabiensis,* 4.5% *An. gambiaes.s,* 0.5% *An.coustani*and 20% *An. quadrianulatus*while 2.5% of the samples could not be identified because DNA was not amplified. Most (66.1%) of those identified as *An. arabiensis* had emerged from dry season samples while all *An. quadrianulatus*and *An. coustani* were from rainy season samples (Table 5).

4. Discussion

An understanding of the spatial and temporal distribution of Anopheles mosquito larva and its determinants is a pre-requisite for devising realistic and sustainable malaria vector control methods that targets immature mosquito stages. This study investigated and therefore reports the spatial and temporal distribution of Anopheles mosquito larva and its determinants in a low and high malaria transmission setting in Tanzania.

Human population distribution and variations in environmental conditions have been known to affect the spatial variations in vector-borne disease occurrence and distributions (Ernst et al., 2006). In this study, a known malaria high transmission site hosted most of the identified mosquito breeding sites as compared to the known low transmission site. Furthermore, in both transmission settings, potential breeding sites were observed more during rainy seasons, this has also been reported in other studies elsewhere (Koenraadt et al., 2004; Akram et al., 2009; Aigbodion and Uyi, 2013).

In the present study breeding sites with positive *Anopheles* mosquito larvae were found to be rice paddies, ditches, containers, swamps, brick pits, foot print and tyre tracks. Interestingly *Anopheles* larvae (mostly *An. arabiensis*) were also found in septic tanks/pits (polluted water) during rainy and dry season. This observation signifies that *Anopheles* mosquitoes are expanding their niches to polluted habitants, a finding which is in line with what has been reported from other studies which that found that polluted breeding sites were positive for *Anopheles* larvae (Sattler et al., 2005; Minakawa et al., 2001; Awolola et al., 2007). This observation highlights the need to consider polluted breeding sites in the control of *Anopheles* mosquitoes through environmental manipulation or modification.

As also reported elsewhere, this study found that most of the potential breeding habitats were manmade in origin and had higher larval density than natural habitats (Djamouko-Djonkam et al., 2019; Aik et al., 2019), this highlights the potential of public health education in controlling mosquito borne diseases by advocating avoidance on creating potential breeding sites. But also, most of the manmade breeding sites tend to cluster around human settlement as observed in this study that more than three quarter of all the potential breeding sites were within less than 100 m from human settlements and they were more likely to have high larval density as compared to those located more than 100 m from human dwelling. This observation could be because of the fact that mosquito reproduction very much depends on the availability of blood from which they derive important nutrients for their developing eggs, therefore breeding sites close to human settlement are likely to have high larval density as mosquito have regular access to human who act as their source of blood meal. The proximity of these highly infested breeding sites to human settlement has control implication, as such breeding sites can easily be located, and since they are close to human settlement it is easy to implement larva source management by engaging the community in an attempt to control mosquito borne infections such as Malaria and lymphatic filariases. It has been argued that larval control by using bio-insecticides or through environmental modification will become efficient and cost effective when the targeted habitats are clustered or when the targeted area size is limited, as observed in our study (Utzinger et al., 2001; Killeen et al., 2002; Fillinger et al., 2003).

Malaria is traditionally considered as a rural disease in Africa, as suitable Anopheles mosquito breeding sites are few in highly populated urban areas (Awolola et al., 2007; Keating et al., 2004; Gardiner et al., 1984). Furthermore, *Anopheles gambiae* sensu stricto, the major African malaria vector is known to preferably breed in temporary clean and clear water (Service MW, 1971). Interestingly, our study found out that, 18.8% water bodies that were positive for *Anopheles* larvae were polluted with either human or animal excreta, but also, it was observed that, high proportion of polluted water habitats had high density of Anopheles larvae as compared to clean water habitats. This observation signifies a shift in the Anopheles mosquito breeding sites preference posing a threat of increased vectorial potential and transmission threshold of malaria in urban areas where polluted water bodies are predominant. Our findings are somehow similar to findings from other studies, where they observed that Anopheles species bread in gutters and other polluted water bodies in Nigeria (Adeleke et al., 2008; Okogun et al., 2005).On PCR analysis, this study found that, most of the identified larva were *An. arabiensis,* followed by *An. gambiaes.s,* both species have been reported to breed in organically polluted water like what is reported in the present study (Sattler et al., 2005; Azrag and Mohammed, 2018; Mukhtar et al., 2003; Afrane et al., 2004).

It is worthwhile to consider algae cover as well as grass cover as important factors that may affect Anopheles spp. Larval ecology as they are known to affect larva population (Service MW, 1977). In line with this, another pressure that could be linked to a specific habitat is vegetation cover, whose impact may be spatially dependent (Mwangangi et al., 2007a; Minakawa et al., 2005; Shililu et al., 2007). Our study found that breeding sites surrounded with short grasses were more likely to have high larval density compared to those with a combination of short and long grasses. Short grasses some of which floating on the aquatic habitat, could serve as a source of nutriment to the larva and therefore explain the high larva density observed in these habitats, this has also been reported in another study elsewhere (Shililu et al., 2007). But also, *Anopheles gambiae* complex is a typical r-strategist, exploiting the increased resources of warmer, open habitats that tend to produce more algae than do shaded habitats (Gimnig

et al., 2002). Surprisingly, in our study we found that habitats in shaded and partial sunlight were significantly more likely to have high density of mosquito larvae than habitats in full sunlight.

Our finding that most of the breeding sites were observed during the rainy season with most of them being rice paddies suggests that larval control may be more effective if implemented during the dry season when the larval habitats are not so wide spread, and during the wet season larval control can be targeted to aquatic habitats in farmlands (Rice paddies) (Minakawa et al., 2005; Fillinger et al., 2004). It has been reported that rice plantations are major mosquito-breeding habitats and constitute the most important factor determining aggregated distribution of *Anopheles* mosquitoes along irrigation canals (e.g. rice irrigation scheme) (Mwangangi et al., 2007b; Kweka et al., 2009). Other studies also found that households which were near mosquito temporary or permanent breeding sites had exhibited higher mosquito abundance (Konradsen et al., 2003; Lindsay et al., 2003; Cano et al., 2006).

This study presents certain limits. Identification of mosquito breeding sites in the study areas was not done on comparable land surface sizes in the two study settings and were not randomly selected; this might have impacted on our findings as the selected sites may have non-representative biotic and abiotic determinants to other breeding sites which were not selected. But also, the use of only two time points to assess temporal variation might have missed important pattern in between the two seasons. Furthermore, the fact that water pollution was only assessed by visual inspection might have missed other pollutants which are not visible to naked eyes, which affect mosquito breeding characteristics.

5. Conclusion and recommendation

This study has found that there is remarkable variation on the spatial and temporal distribution of Anopheles mosquito breeding sites. Type of water, distance from the breeding site to human settlement, light intensity and habitat origin were significant predictors of this distribution. With increased global emphasis on control measures that targets mosquito immature stages; we recommend that larval control measures should be developed while considering the findings from this study. But also further studies on Spatial and temporal distribution of *Anopheles* mosquito's larvae, should consider polluted water as potential breeding sites for *Anopheles* mosquitoes.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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