

Mosquito larvae abundance and adult productivity in an area of intensive malaria control in northeast Tanzania

Eliningaya J. Kweka ^{1, 2, *}, Frank Magogo ³, Filemoni Tenu ³, Ming-Chieh Lee ⁴, William N. Kisinza ³, Leonard E.G. Mboera ⁵⁺

¹ Department of Medical Parasitology and Entomology, School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania, ² Tanzania Plant Health and Pesticides Authority, Department of Pesticides Management, Pesticides Bioefficacy Section, P.O. Box 3024, Arusha, Tanzania, ³ National Institute for Medical Research, Amani Medical Research Centre, P.O. Box 81, Muheza, Tanzania, ⁴ Program in Public Health, College of Health Sciences, University of California, Irvine, CA 92697-4050, USA, ⁵ National Institute for Medical Research, Headquarters, P.O. Box 9653, Dar es Salaam, Tanzania

ABSTRACT

Introduction: Targeting mosquito larval stages for malaria vector control is gaining more popularity in sub-Saharan Africa. Here we assessed mosquito species diversity and the effects of physicochemical parameters, distance to human habitation, and land use types on the productivity of larval habitats, with a focus on Anopheles gambiae (sensu lato).

Methods: A total of 21 habitats were surveyed for 15 weeks to evaluate species-specific oviposition preferences and species composition of *An. gambiae* (*s.l.*). Habitats were classified as drainage ditches, swamps, and man-made wells, whereas land types were classified as shrubs, farmland, and pasture. The distance to the nearest house and the habitat's physical characteristics and physicochemical parameters were recorded.

Results: A total of 4511 mosquito larvae were sampled during the survey period. Of these, 54.93% were An. *gambiae* (s.l.), 2.09% were An. *funestus*, and 42.98% were Culex quinquefasciatus. Among 2397 larvae of Anopheles mosquitoes sampled, 501 were identified to the species level using the TaqMan genotyping assay. A total of 251(50.10%) Anopheles specimens identified were An. arabiensis, 173(34.53%) were An. gambiae (s.s.) and 17(3.39%) were An. coustani; DNA was not successfully amplified for 60 (11.98%) An. gambiae (s.l.).

Conclusion: The presence of *An. gambiae* larvae was positively correlated with habitats located nearest to houses.

Keywords: Land-use; Larval abundance; Productivity; Anopheles gambiae (s.s.); An. arabiensis; An. funestus; Tanzania

Introduction

Land use changes in different areas have influenced the availability of potential mosquito larval habitats (Bezirtzoglou et al., 2011; Bueno-Mari & Jimenez-Peydro, 2013). Understanding larval habitat abundance in different land use types is critical for the management of mosquito oviposition sites. In western Kenya, targeted larvicide applications have contributed to decreased entomological inoculation rates (Fillinger et al., 2009; Kweka et al., 2011b; Ndenga et al., 2011).

Larval source management (LSM) has long been used in malaria control, although it has been practiced on a small scale (Imbahale et al., 2010; Kweka et al., 2012), particularly in tropical Africa. Effective LSM is a priority in reducing vector populations because of the following reasons: (i) increase

*Corresponding author: E-mail address: pat.kweka@gmail.com

⁺ Deceased, May God Rest the Deceased in Piece, AMEN



of insecticide resistance for insecticide classes used in indoor residual spraying (IRS) or insecticidetreated mosquito nets (ITNs) (Padonou et al., 2012b; Okumu et al., 2013; Osse et al., 2013); (ii) changes in feeding and resting behaviour of adult mosquitoes due to widespread use of intervention tools (Padonou et al., 2012a); (iii) 'refuges' of vector populations through alternative outdoor blood-meal sources (Coetzee et al., 2000; Kweka et al., 2011b; Sinka et al., 2012); and (iv) LSM suppresses mosquito populations at their production site.

Effective utilization of LSM needs appropriate information and knowledge on land use types, larval abundance, and gravid female mosquito response to habitat resources (Ndenga et al., 2011; Kweka et al., 2012; Ndenga et al., 2012). Some human activities such as deforestation in the Muheza District have led to a substantial increase of areas better suited to *Anopheles gambiae* (s.l.). These open habitats provide more suitable mosquito oviposition sites and increase the risk of malaria transmission (Lindsay et al., 2000; Balls et al., 2004; Bødker et al., 2006). Despite the intensive research activities in the area, there is limited information on habitat larval density and productivity. Habitats in different land use types frequently show the existence of different larval densities (Munga et al., 2006a; Kweka et al., 2011b, 2012). This is, at least partially, caused by the oviposition site preferences of mosquito species (Bentley & Day, 1989). The preference of *An. gambiae* (s.l.) gravid females to specific habitats might be influenced by several of the following factors; food sources for larvae; habitat characteristics (pH, grass cover, and turbidity); organic pollutants (McCrae, 1984); predators (Kweka et al., 2011a); olfactory cues (Bentley & Day, 1989; Rejmánková et al., 2005); and debris and algae (Rejmánková et al., 1996; Kweka et al., 2011b). Mosquitoes of the *Anopheles funestus* group show a preference for wide habitats with vegetation cover such as swamps (Nambunga et al., 2020; Debrah et al., 2021).

This study was carried out to determine the larval abundance and adult productivity of several human-biting mosquito species in different habitat and land use types. Additionally, we determined habitat characteristics, physicochemical parameters, and the distance to the nearest houses. Until 2005, when the use of ITN was scaled-up, this region was considered a holoendemic area for malaria (WHO, 1975; Gillies & Coetzee, 1987). The objective of this study was to identify characteristics of productive larval habitats about distance to human dwellings to aid future community-wide larvicide efforts. In addition, we verified the species composition compared to historical data for the same area of Muheza (Kabula et al., 2011, Kweka et al., 2008).

Materials and methods

Study sites

The present study was conducted in Muheza District, Northeast Tanzania (Figure 1). Productive larval habitats were searched in different land use types in Muheza peri-urban areas using standard dipper methods. Twenty dips were made per habitat to estimate the larval abundance. For large water bodies, 20 dips were made randomly around the whole water body. Larvae were identified to the species level using a morphological key (Gillies & Coetzee, 1987). Twenty-one productive oviposition habitats containing larval stages of *Anopheles gambiae* (*s.l.*) were chosen. These twenty-one habitats were the only positive habitat types in which *Anopheles* spp. could be observed: swamps; drainage ditches; and man-made wells (larval habitats such as water pits as described by Munga et al., 2007). The habitats occurred within three different land-use types: pastureland, farmland and shrubland; these were shown in previous studies to represent productive habitat types (Munga et al., 2006a, b; Kweka et al., 2011b). Habitat positions were marked with labelled pegs and georeferenced using a handheld Garmin Global Positioning System unit (eTrex Venture HC, USA).





Figure 1: A map showing the study sites and habitat types examined during the study period in Muheza, Tanga Region, Tanzania.

Land use and habitat type definitions

Land use definition was based on ongoing activities; shrubland was considered as land covered by short vegetation and not used for agriculture or grazing; livestock grazing areas were considered as pastureland; and land used for crop production was considered as farmland (Munga et al., 2006b). The definition and classification of habitat types in the present study considered the categories established by a previous study (Minakawa et al., 2005). The habitat types used were swamp, man-made wells and drainage ditches.

Mosquito sampling and identification

A standard dipper (350 ml, BioQuip Products, Inc. California, USA) was used for weekly sampling of mosquito larvae and pupae (WHO, 1975). All mosquito larvae were immediately stored in absolute ethanol in separate vials after being identified to the species level according to the morphological key developed by Gillies & Coetzee (1987). Vials with larvae were kept at 4 °C until molecular identification. Pupae were kept separately in 250 ml paper cups filled with 200 ml of water from their original habitat. After emergence, individual adults were morphologically sorted according to sex and identified to the species level (Gillies & Coetzee, 1987). Anophelines (both larvae and adults) were further molecularly identified to the species level, utilizing the TaqMan single nucleotide polymorphism genotyping protocols (Bass et al., 2007; Walker et al., 2007).

Larval habitat characterization

We measured the depth, length and width in centimetres of the habitats weekly. The following parameters were also measured weekly from 8:30 h to 13:00 h during larval sampling: temperature; pH; conductivity, turbidity; and chlorophyll content. A Hanna[®] Instruments portable pH meter (SN 06592887; Woonsocket, RI, USA) was utilized for the registration of temperature, pH and conductivity. Chlorophyll and turbidity were determined with a portable turbidimeter (AquaFluor[,] Sunnyvale, CA, USA). Both instruments were calibrated fortnightly for accurate reading using the manufacturer's instructions.



Predator quantification and identification

Invertebrates found in the habitats during sampling were identified using the key by Needham & Needham (1941). The density of invertebrate predators was counted for 20 dips per habitat.

Habitat grass cover estimation

Grass cover was roughly estimated in percentages with a scale interval of 5%. Grass cover is defined as the percentage of habitats covered by emergent and/or floating vegetation. Sampling was done weekly on every Tuesday. Grass cover estimation has been used in previous studies and shown to be a good method for establishing the relationship between vegetation cover and larval abundance (Munga et al., 2009; Kweka et al., 2011b, 2012).

Abundance of larval stages and species diversity

Larval abundance was defined as the average number of aquatic stages of mosquitoes found in 20 dips (Munga et al., 2006b; Mutuku et al., 2006; Kweka et al., 2011b, 2012). The pupae sampled from each habitat were kept in paper cups with water from the same habitat; pupae were held until emergence. Habitat productivity was defined as the number of emerging adult female mosquitoes per square meter (Munga et al., 2006b; Kweka et al., 2011b). (The male mosquitoes' productivity was not considered as they are not disease vectors).

A diversity index was used to assess mosquito species composition and abundance. Diversity indices provide more detailed information about community composition than simply species richness. The Simpson diversity index is a quantitative measure reflecting both the number of species and individual species abundance. The value of a diversity index increases both when the number of species increases and when the evenness of their abundance increases. For a given number of species, the value of a diversity index is maximized when all types are equally abundant. Species diversity index (D) was computed using the Simpson's diversity index (Alatalo and Alatalo, 1977; Norris and Pollock, 1998) for all 15 weeks in different land use and habitat types using the formula:

$$D = \sum_{i=1}^{N} \left(P_{i}^{2} \right)$$

where p_i is the relative abundance of the i-th species and N is the total number of species. D varies from 0 to 1, with values near zero corresponding to highly diverse or heterogeneous communities and values near one corresponding to more homogeneous ecosystems. When the D value is small, there is a dominant species (in terms of individual species); when D is larger, there is low species dominance.

Stepwise multivariate logistic regression analysis with backward elimination of variables was used to assess the strength of the association between mosquito larval abundance and land use types, grass cover percentage, habitat types, conductivity, chlorophyll level, temperature, turbidity, predator species abundance, and distance to the nearest house (less than 100 meters or more than 100 meters from household). Data analysis was performed using Stata version 12.1 (STATA Corp.2012, Texas, USA) and PWAS statistics version 26 (SPSS Inc., Chicago, IL, USA).

Results

Larval abundance and diversity

Larval habitats were found in all three land use types, i.e. farmland, shrubland and pastureland. A total of 4511 mosquito aquatic stages were sampled during the survey period. Of these, 4363 (96.72%) were larvae and 148 (3.28%) were pupae. From the sampled larvae, 2397 (54.94%) were An. gambiae (s.l.), 91 (2.09%) were An. funestus, and 1875 (42.98%) were Culex quinquefasciatus. Among all pupae sampled, 121 (81.76%) were An. gambiae (s.l.), and 27 (18.24%) were Cx. quinquefasciatus.



Based on habitat types considered in this study, a total of 696 larvae were sampled from two swamps; of these, 85 (12.2%) were An. gambiae (s.l.), and 611 (87.8%) were Cx. quinquefasciatus; An. funestus was not found in either swamp studied. In man-made wells (n = 18), a total of 3536 larvae were sampled; of these, 2215 (62.64%) were An. gambiae (s.l.), 89 (2.52%) were An. funestus, and 1232 (34.84%) were Cx. quinquefasciatus. A total of 121 pupae of An. gambiae (s.l.) were sampled from the man-made wells but no Cx. quinquefasciatus pupae were found during the study period. From the only studied drainage ditch, 131 larvae were sampled; of these, 97 (74.05%) were An. gambiae (s.l.), 2 (1.53%) were Cx. quinquefasciatus, and 32 (24.43%) were An. funestus. No pupae were sampled from the drainage ditch. In the shrubland, a total of 883 larvae were sampled; of these, 681 (77.12%) were An. gambiae (s.l.) and 202 (22.88%) Cx. quinquefasciatus and no An. funestus was found in drainage ditches. A total of 28 pupae of Cx. quinquefasciatus were sampled from shrubland. In pastureland, 1599 larvae were sampled; of these, 605 (37.84%) were An. gambiae (s.l.), 2 (0.13%) were An. funestus, and 992 (62.0%) were Cx. quinquefasciatus; 3 pupae of An. gambiae (s.l.), 89 (4.74%) were An. funestus, and 681 (36.20%) were Cx. quinquefasciatus.

Species diversity varied between sampling weeks (Figure 2). In habitat types, swamps had a diversity index (DI) of 0.21, man-made wells 0.48 and drainage ditches 0.39. This indicates a high heterogeneity among species. When data was categorised by land use types, a high heterogeneity was observed among species (pasture DI = 0.21, shrub lands DI = 0.38; farmland DI = 0.39).



Figure 2: Simpson's diversity index for mosquito larvae during the survey period of 15 weeks.

Among 2397 larvae of *An. gambiae* (*s.l.*) collected, 501 were chosen for further molecular identification to the species level (Walker et al., 2007) as resources were limited for testing all specimens sampled. The selected proportion was selected regarding total samples collected weekly. A total of 251 (50%) specimens were identified as *An. arabiensis* (indicated in blue in Figure 3), 173 (35%) were identified as *An. gambiae* (*s.s.*) (indicated in red in Fig. 3), and 17 (3%) were *An. coustani* (Fig. 3). DNA amplification was not successful for 60 (12 %) specimens. The overall habitat productivity was found to be 0.004 adults/m²/week for *An. gambiae* (*s.l.*) and 0.002 adults/m²/week for *Cx. quinquefasciatus*.





Figure 3: Allelic species discrimination results by TaqMan single nucleotide polymorphism genotyping for *Anopheles gambiae* (s.s.) (red), *An. arabiensis* (blue) and other *Anopheles* spp. (green squares) larvae sampled in Muheza, North-East Tanzania for 15 weeks. Controls are indicated by light green triangles.

Habitat types and larval abundance

The three habitat types studied here were swamps, drainage ditches and man-made wells. The larval abundance of *An. gambiae* was significantly associated with man-made wells when adjusted for other variables (Table 1 Factors associated with the presence of aquatic stages of *Anopheles gambiae s.l.* for both crude and adjusted odd ratio); the larval abundance of *An. funestus* as assessed separately because there were habitats without *An. funestus* larvae (Table 2Factors associated with presence of aquatic stages of *Anopheles funestus* for both crude and adjusted). The larval abundance of *Cx. quinquefasciatus* was significantly associated with the man-made wells and swamps, but not with drainage ditches as crude OR when adjusted for covariables (Table 3 Factors associated with presence of aquatic stages of Culicine species for both crude and adjusted odds ratio).

Volume 26: Issue 3, April 20 Crude Prvalue			95% 95%	C. I.	https://d	<u>'g/10.4314/thrb.v</u> 95% C. I.		
Parameter	OR		Lower	Upper	Adjusted OR		Lower	Upper
Habitat type								
Swamp	1	-	-	-	1	-	-	-
Man-made well	2.04	0.277	0.56	7.43	0.13	0.035	0.02	0.87
Drainage system	1.45	0.667	0.26	8.01	0.13	0.075	0.01	1.22
Conductivity (<mark>µS/cm)</mark>	6.45	<0.001	3.50	11.90	0.83	0.780	0.22	3.14
рН	1.52	<0.001	1.27	1.81	1.39	0.146	0.89	2.16
Temperature (°C)	1.11	<0.001	1.06	1.15	1.02	0.788	0.90	1.14
Grass cover (%)	1.01	0.225	1.00	1.01	0.99	0.030	0.97	1.00
Land use								
Pasture	1	-	-	-	1	-	-	-
Shrub	0.91	0.855	0.32	2.56	3.57	0.134	0.67	18.96
Farmland	3.92	<0.001	1.82	8.45	14.01	<0.001	3.70	53.04
Chlorophyll (µg/L)	1.68	0.092	0.92	3.08	0.52	0.144	0.21	1.25
Turbidity (NTU)	77.41	<0.001	21.74	275.61	69.88	0.007	3.12	1564.10
Presence of predators								
No	1	-	-	-	1	-	-	-
Yes	1.52	0.336	0.65	3.55	0.73	0.526	0.27	1.96
Distance to house in meters								
More than 100m	1	-	-	-	1	-	-	-
100m or less	2.20	0.003	1.29	3.73	1.70	0.182	0.78	3.71

	Crudo	Byaluo	95% C. I.			Pavaluo	95% C. I.	
Associated factors	OR	r-value	Lower	Upper	Adjusted OR	r-value	Lower	Upper
Conductivity (<mark>µS/cm)</mark>	1.66	0.117	0.88	3.13	0.56	0.569	0.08	4.14
рН	1.77	0.070	0.95	3.29	1.62	0.293	0.66	3.95
Temperature (°C)	1.09	0.047	1.00	1.18	1.06	0.636	0.84	1.32
Grass cover (%)	1.01	0.218	0.99	1.02	1.01	0.423	0.99	1.03
Chlorophyll (µg/L)	2.06	0.158	0.75	5.64	1.49	0.552	0.40	5.56
Turbidity (NTU)	3.48	0.068	0.91	13.30	5.21	0.370	0.14	192.86
Presence of predators								
No								
Yes	10.96	<0.001	3.92	30.61	8.65	<0.001	2.93	25.56
Distance to house in meters								
More than 100m								
100m or less	1.50	0.42	0.56	4.01	1.06	0.92	0.34	3.34

** Note: In some levels of habitat and land use types do not have Anopheles funestus. So they were omitted as comparison could not be possible.



Grass cover and larval abundance

Increase in percentage grass cover was found to result in significantly decreased larval abundance of *An. gambiae* (*s.l.*) only when adjusted for other parameters. The decrease in productivity was not statistically significant when grass cover was considered alone (crude odds ratio, OR; Tables 1) while for *An.funestus* the association with grass cover was not statistically significant (Table 2). In contrast, the larval abundance of *Cx. quinquefasciatus* increased significantly with vegetation cover when correlated singly; when adjusted for other variables, the vegetation cover was found to not affect larval density (Table 3).

Land use and larval abundance

The three land use types characterised in this study were farmland, pastureland and shrubland. Larval abundance of *An. gambiae* (*s.l.*) was positively associated with farmland (Table 1), whereas that of *Cx. quinquefasciatus* was significantly positively associated with shrubland but not with farmland (Table 3). In the farmland, maize (*Zea mays*) was the dominant crop in all areas, with other crops being paddy, vegetables and cassava.

Physicochemical characteristics and larval abundance

In *An. gambiae* (*s.l.*), the increase in larval abundance was significantly associated with a unit increase of conductivity, pH, and temperature when considered singly, but not when adjusted for other parameters (Tables 1) while for *An.funestus* the only temperature on unadjusted odds ratio was significantly associated with larval abundance (Table 2). Importantly, turbidity was associated with the increase of *An. gambiae* (*s.l.*) but not for *An. funestus* larvae (Tables 1 and 2). In *Cx. quinquefasciatus*, the increase in conductivity and pH were significantly associated with an increase in larval abundance (Table 3). Both turbidity and temperature were significantly associated with *Cx. quinquefasciatus* larval abundance both about crude and adjusted OR

		P-value	95% C. I.			P-value	95% C. I.	
Associated factors	Crude OR	i value	Lower	Upper	Adjusted OR	1 value	Lower	Upper
Habitat type								
Swamp								
Man-made well	0.29	0.024	0.10	0.85	0.50	0.340	0.12	2.07
Drainage system	0.24	0.072	0.05	1.13	0.28	0.184	0.04	1.85
Conductivity (<mark>µS/cm)</mark>	3.40	<0.001	1.96	5.88	0.45	0.324	0.09	2.19
рН	1.38	<0.001	1.20	1.58	0.90	0.608	0.59	1.36
Temperature (°C)	1.11	<0.001	1.07	1.17	1.17	0.007	1.04	1.31
Grass cover (%)	1.02	<0.001	1.01	1.03	1.01	0.140	1.00	1.02
Land use								
Pasture								
Shrub	0.07	<0.001	0.02	0.24	0.08	0.002	0.02	0.41
Farm land	0.62	0.120	0.34	1.13	0.54	0.176	0.22	1.32
Chlorophyll (µg/L)	0.88	0.709	0.47	1.68	0.56	0.181	0.24	1.31
Turbidity (NTU)	20.32	<0.001	6.49	63.66	30.31	0.040	1.16	792.42
Presence of predators								
No								
Yes	2.78	0.017	1.20	6.47	2.30	0.087	0.89	5.97
Distance to house in meters								
More than 100m								
100m or less	0.70	0.16	0.43	1.15	1.22	0.60	0.57	2.62



ratios (Table 3). Chlorophyll levels were not significantly associated with larval abundance in any of the species, using crude or adjusted OR (Tables 1, 2 and 3).

Habitat distance to the nearest house and larval abundance

The habitat distance to the nearest house was significantly associated with an increase in *An. gambiae* (*s.l.*) larval abundance when analysed singly but not when adjusted for covariables. When treated singly, the probability of having more larvae of *An. gambiae* (*s.l.*) was 2.2 times in habitats close to the houses (distance to the nearest house \leq 100 m); when adjusted for other variables, this probability decreased to 1.7 times and was not significant (Table 1). In *An. funestus*, when the distance from habitats to the nearest house was treated singly, the crude OR was 1.5 times greater than for habitats located at > 100 m from houses. However, when adjusted for other parameters, the larval abundance of *An. funestus* decreased to 1.06 times when compared with the habitats located at > 100 m from houses (Table 2). However, sample size, i.e. the number of *An. funestus* found was consistently very low. In *Cx. quinquefasciatus*, the larval abundance had no significant association with the distance to the nearest house in crude odds ratio or when adjusted (Table 3). The larval abundance for all species was not associated with livestock abundance as there were no animal sheds around the considered houses.

Predators and larval abundance

The identified predators were tadpoles, backswimmers (Notonectidae), *Gambusia affinis*, *Belestoma* and dragonfly nymphs (Odonata). These were larval mosquito predators elsewhere in natural mosquito oviposition habitats and in confined experiments (Kweka et al., 2011b, 2012). Counts of individual predators in mosquito oviposition sites were low and were therefore pooled together irrespective of species. In *An. gambiae* (*s.l.*), the density of predators had no significant association with larval mosquito abundance (both crude and adjusted OR; Table 1). The larval abundance of *An. funestus* was significantly positively associated with predator density (both crude and adjusted OR; Table 2). The high abundance of *Cx. quinquefasciatus* was associated with a significant reduction of predator abundance (crude OR), but the association was not significant when adjusted for covariables (Table 3).

Discussion

The objective of this study was to determine larval abundance and adult productivity of mosquito species in different habitat types under different land use types in Muheza District, Tanga Region, northeastern Tanzania. The study in addition determined habitat characteristics, physicochemical parameters and distance to the nearest houses. Among different land use and habitat types, farmland and man-made wells had the highest mosquito larval abundance. This is similar to findings in western Kenya (Kweka et al., 2011b, 2012; Munga et al., 2006b, 2009; Ndenga et al., 2011). Larval abundance of *An. gambiae s.l.* was found to be highest in farmland, followed by pastureland and lowest in shrubland. The highest larval abundance in farmland is likely to be associated with the crop type cultivated in crude odds ratio and adjusted analysis. Where the main crop on farmland was maize, habitats had high larval and pupal densities. Maize pollens have been described as an important food source for anopheline mosquitoes in Kenya (Kweka et al., 2012) and in Ethiopia (Ye-Ebiyo et al., 2000, 2003a, b). The high density of larvae in farmland habitats may be associated with food availability, as well as with the nearness of farmlands to housing. This means that the adjusted odds ratio has shown that, the combination of all variables could not change the fact that, farming land has more larvae in habitats than other land types.

Vector species composition in this study revealed higher proportions of *An. arabiensis* than *An. gambiae* (s.s.). In previous studies during the 1970s, this area was predominantly occupied by *An. gambiae* (s.s.) (White & Magayuka, 1972; White, 1974). In later studies conducted in the 1990s, the mosquito population had shifted from *An. gambiae* (s.s.) to *An. arabiensis* following massive distributions of ITNs and IRS coverage started in the early 1990s (Mnzava et al., 1995; Magesa et al.,



1991; Maxwell et al., 2002; Curtis et al., 2006; Kitau et al., 2012; Kweka et al., 2020). The previous studies found *An. gambiae* (s.s.) to occur at a proportion of 41.9% vs 39.1% for *An. arabiensis*. In the following survey, the proportion of *An. gambiae* (s.s.) was found to be 7.7% while that of *An. arabiensis* had doubled (Derua et al., 2012). *Anopheles gambiae* (s.s.) is an endophilic and endophagic species (Futami et al., 2014; Iwashita et al., 2014). The long-term suppression of the human-feeding population through LLINs use and the exciton-repellence effect of LLINs have caused an elevated mortality in *An. gambiae* (s.s.) due to having contact with insecticides and partial or no blood-meal access (Mutuku et al., 2006). A similar trend was reported in western Kenya where the LLINS have been used for decades (Bayoh et al., 2010) and in Tanzania (Kitau et al., 2012; Kreppel et al., 2020). The population increase in *An. arabiensis* in our study in Muheza district could be attributed to the following factors: (i) availability of bovines as blood-meal sources for *An. arabiensis*, which has been observed in other sites with the same report of *An. arabiensis* increase (Bayoh et al., 2010); and (ii) *An. arabiensis*, which is primarily zoophilic and exophagic, cannot be targeted easily with LLINs and IRS.

The pH of habitat water was positively associated with the larval abundance of both *An. gambiae* (*s.s.*) and *Cx. quinquefasciatus* but not of *An. funestus when analyzed* in crude odds ratio but when odds ratio was adjusted with other variables none of the species was correlated positively with habitat water pH. Previous studies showed that the abundance of larvae of *An. gambiae* (*s.l.*), *Cx. quinquefasciatus* and *An. funestus* were not associated with pH (Kweka et al., 2012; Ndenga et al., 2012), but a study conducted by Emidi et al. (2017) in this study area showed that anopheline larvae decreased with increase in habitat water pH. A previous study in western Kenya showed an increase in larval abundance two weeks after fertilizer application, the habitats became clear by increased decomposition and sedimentation of debris. This influences positively the abundance of *An. gambiae* (*s.l.*) larvae whereas four weeks after application, the sedimentation rate of materials increases organic matter pollutants which in turn supports the survivorship of aquatic stages of *Cx. quinquefasciatus* (Victor & Reuben, 2000; Mutero et al., 2004; Muturi et al., 2007; Mwangangi et al., 2006a, b, 2007). The causes of pH variations during the cropping season are unknown since farmers of the studied region do not use synthetic fertilisers.

In the present study, the conductivity of habitat water were found to be associated with the abundance of *An. gambiae* (*s.l.*) and *Cx. quinquefasciatus* aquatic stages but not of *An. funestus* in both crude and adjusted odds ratio while for conductivity, the crude odds ratio was associated with abundance of *An. funestus* and *An. gambiae* s.l. while adjusted odd ratio had no such influence. The *An. funestus* abundance could not be influenced with either crude or adjusted odds ratio of habitat water conductivity. In a previous study in Mwea, rice irrigation schemes in central Kenya, it was found that in sunlit ephemeral habitats *An. gambiae* (*s.l.*) survived better with higher conductivity and turbidity. It is not well understood why *An. funestus* larvae seem not to be associated with a decrease or increase in turbidity or conductivity. Similar observations have been reported from studies elsewhere (Kweka et al., 2011b; Ndenga et al., 2012). One reason could be the comparatively small number of *An. funestus* found, which may be challenging to identify statistically significant associations.

In contrast to a previous report (Kweka et al., 2011b), the present study did not find any strong association between larval abundance (*Cx. quinquefasciatus, An. gambiae* s.l. and *An. funestus*) and habitat chlorophyll concentrations in both crude and adjusted odds ratio. This might be the results of the short study period of 15 weeks (one rainy season) compared to other studies which took up to two years (four seasons; rainy, short-rainy, dry, and semi-dry seasons of the year, repeated twice) of monitoring habitat physicochemical parameters and larval abundance (Kweka et al., 2011b, 2012).

In the present study, the increase in one degree Celsius was found to be associated with an increase in larval abundance by 1.01, 1.11 and 1.09 for *An. gambiae* (s.l.), *Cx. quinquefasciatus* and *An. funestus*, respectively. Temperature is a key factor for the development of mosquito aquatic stages (Lyons et al., 2013). In other field studies, *An. funestus* larval abundance in swamps was found to be positively correlated with an increase in temperature (Kweka et al., 2012). In other laboratory findings,



both *An. gambiae* (*s.l.*) and *An. funestus* have shown a similar trend of aquatic stages development and growth period length to be governed by temperature (Bayoh & Lindsay, 2004; Lyons et al., 2013). In *Cx. quinquefasciatus*, an increase of temperature from 16 °C to 24 °C resulted in a 2.9-fold shortening of the developmental time (Ciota et al., 2014). Usually, insect aquatic life stage development is directly proportional to an increase in temperature (Bayoh & Lindsay, 2004).

The increase in percentage of grass cover was positively associated with the abundance of aquatic stages of *Cx. quinquefasciatus*, but not of *An. gambiae* (*s.l.*) or *An. funestus*. This study is contrary to previous studies reported that *An. funestus* preferentially breed in shaded habitats with high grass cover (Wamae et al., 2010; Kweka et al., 2011b). In this study area it was observed that, the more the percentage cover of grasses, the more decomposing organic which supports the culec oviposition attractation and larvae abundance.

In terms of land use types, *An. gambiae* (*s.l.*) larval abundance was associated with farmland, while *Cx. quinquefasciatus* abundance was associated with shrubland. The abundance of *An. gambiae* (*s.l.*) in farmland was similar to what was reported previously in Ethiopia and Kenya (YE-ebiyo et al., 2003b; Kweka et al., 2012). Shrubland, where *Cx quinquefasciatus* larvae were most abundant, is associated with low sunlight exposure and high levels of organic pollutants. The positive association fits with known preferred oviposition sites of *Cx. quinquefasciatus* (Ansari et al., 2004; Impoinvil et al., 2008). Most of these habitats in the Muheza region are not permanent, but only last for a short period after the long rainy season. This is different to other areas, where habitats are frequently permanent and inhabited by larvae throughout the year (Kweka et al., 2011b, 2012; Mala & Irungu, 2011; Ndenga et al., 2012)

The presence of predators influenced mosquito species abundance differently. In this study the findings showed that the predator density did not influence the abundance of *An. gambiae* (*s.l.*). This is likely due to the generally low abundance of predators for the majority of studied habitats. Gravid females may actively avoid habitats occupied by predators as shown in laboratory studies (Munga et al., 2006a; Warburg et al., 2011). In contrast, the larval abundance of *An. funestus* and *Cx. quinquefasciatus* increased with predator density (Kweka et al., 2011a; Warburg et al., 2011).

Studies conducted in West Africa, revealed that predation pressure plays a major role in the adaptation of different *An. gambiae* s.l. species to different habitats (Diabate et al., 2008; Gimonneau et al., 2010; Roux et al., 2014). *Anopheles gambiae* (*s.l.*) was more frequently found in shallow and sunlit ephemeral habitats. Predators might encounter difficulties in colonising these different from long-lasting habitats where *An. funestus* and *Cx. quinquefasciatus* occur. High larval survivorship and subsequently higher productivity of *An. gambiae* (*s.l.*) than *Cx. quinquefasciatus* and *An. funestus* was sometimes reported in the past (Munga et al., 2006); Kweka et al., 2011b; Ndenga et al., 2011). *Anopheles gambiae* (*s.l.*) larvae were also frequently found in higher proportions compared to *Cx. quinquefasciatus* in our study and inhabited even polluted habitats, similar to what has been documented in other African peri-urban areas in other studies (Awolola et al., 2007; Tene Fossog et al., 2013). In Kumasi, Ghana, the polluted habitats in urban vegetable gardens have been found to be potential oviposition sites and colonised with *An. gambiae* (*s.l.*) (Afrane et al., 2004, 2012).

This study demonstrated species heterogeneity during the whole study period in all habitat and land use types. No species was found to dominate throughout the study period. Species variations occurred between habitat types. Seasonality is a major determinant of temporal heterogeneity in the distribution of such habitats and land use types, and consequently contribute to the heterogeneity in the distribution of mosquito aquatic stages (Walker & Lynch, 2007).

The nearest habitats to human dwellings (< 100 m) in our study had higher abundances of An. gambiae (s.l.) aquatic stages than those further away (\geq 100 m). Other studies similarly showed that An. gambiae (s.s) is anthropophilic and utilizes those habitats near human dwellings for oviposition (Minakawa et al., 2002). This might be an evolutionary optimization to reduce flight time and thus costs of energy and predation.

Anopheles gambiae (s.l.) habitat productivity in the study area was found to be twice as high as that of Cx. quinquefasciatus. Yet, 0.0004 An. gambiae (s.l.) females per m^2 is much lower than that



reported in other areas, such as in the western Kenya highlands $(1.8-16.93/m^2)$ (Kweka et al., 2011b; Ndenga et al., 2011). The low productivity of both Cx. *quinquefasciatus* and An. *gambiae* (*s.l.*) possibly reflects a decline of vector and non-vector species reported in the Tanga region for the past two decades (Meyrowitsch et al., 2011; Derua et al., 2012).

Conclusions

The findings of this study show that the abundance of mosquito aquatic stages in Muheza peri-urban areas is composed of higher proportions of *An. arabiensis* as compared to *An. gambiae* (s.s.) and indicates that larval control of *An. gambiae* (s.l.) should prioritize targeting drainage ditches and manmade wells close to human habitation.

Funding

This study was funded by the TASENE project (a collaborative programme of COSTECH, WOTRO and SIDA) granted to EJK as a southern partner (grant number TASENE/Sida/2/2012).

Ethical approval

This study was granted ethical approval by the Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/1584). In the field, individual field owners were consulted for permission to use the habitats in their fields for 15 weeks and were informed about the aim of the study.

CRediT authorship contribution statement.

Eliningaya J. Kweka: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Validation, Writing - original draft, Writing - review & editing, Visualization. **Frank Magogo:** Formal analysis, Investigation, Resources, Writing - original draft, Visualization. **Filemoni Tenu:** Formal analysis, Visualization. **Ming-Chieh Lee:** Formal analysis. **William N. Kisinza:** Writing - review & editing. **Leonard E.G. Mboera:** Writing - original draft, Writing - review & editing, Supervision.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files.

Declaration of competing interests

he authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank Thobias Sekanyika, Irene Semkiwa, Isaya Kibwana and Arno Nkinga for their devotion in field work, mosquito rearing, and experimental work. Jeremiah Mushi is acknowledged for statistical advice. Dr Stephen Munga is acknowledged for critical comments to an earlier version of this manuscript. We particularly thank all community members who granted permission to select habitats within their fields.

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