Identification of Entomological Drivers for Persisting High Malaria Transmission in Ruangwa District Lindi Region Tanzania

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ABSTRACT: *High malaria prevalence remains a major problem, despite high coverage rates of malaria control interventions. The study was carried out to investigate entomological factors responsible for malaria high malaria transmission in Ruangwa district. Three villages were selected from three wards of Ruangwa district; Mnacho and Chienjere. Likangara represented low, Nandagara moderate and Chienjere high malaria prevalence's. In each village six houses with open eaves were selected for mosquito collection both indoors and outdoors using Human Landing Catches. A total of 2532 female mosquitoes were collected. Malaria vectors constituted 26.66 % (An.gambiae s.l 680(26.35); An.funestus 8 (0.31) and An.coustani s.l 3 (0.11%).while non –malaria vectors accounted for 73.26 (Culex sp. 1854 (73.2%) Aedes sp. 1 (0.03%). Most mosquito abundance was dominant in Chienjere 932 (37%), followed by Likangara 820 (31%) and the least was Nandagara composed of 780 (31%). Out of 688 anophelines subjected for PCR speciation, An.gambiae s.s was relatively higher 297 (43%) compared to An.arabiensis 278 (40%), An.funestus s.s 6 (0.87%) and unamplified 107 (16%). The high abundance of Anopheles mosquitoes was observed in rainy season 553 (95.18%) as compared to dry season 28 (4.82%). An.gambiae s.s and An.arabiensis species are responsible for maintaining high prevalence of malaria even in the absence of other vectors. An. coustani has an epidemiological concern since it is important vector in neighboring country of Kenya and Zambia. The findings provide useful information that would enable to plan and innovative and effective malaria control strategies in the district.*

Key words: Malaria prevalence, species, *An.gambiae*, *An.funestus*, *An.coustani*, Ruangwa

Introduction

Globally, the malaria burden has dramatically decreased in the past decade as shown by the 14-percentage fall in malaria incidence rates in all age groups worldwide since 2010 and by 20 percent in the WHO African Region as attested by the World Health Organization (WHO, 2019). The Long-

Lasting Insecticides treated Nets (LLINs), Indoor Residual Spraying (IRS) and case management with artemisinin combination therapies (ACTs) have strongly contributed to this reduction (Ssemppira *et al.,* 2017; WHO,2019). However, malaria continuity in these settings was noted in the World Malaria Report. Conversely, this resilience is mainly linked to the spread of insecticides resistance across many endemic countries (Ranson and Lissenden, 2016). Mosquitoes species responsible for the residual transmission can also comfortably and successful feed and survive on blood from non- human host (Main *et al.,* 2016). Thus, residual malaria transmissions are now persistent in many places (Mwesigwa et *al.,* 2017). Residual transmission refers to those fractions of transmission, mediated by mosquitoes, which are behaviourally resilient to the existing primary malaria interventions. Even though, residual malaria transmission was not given enough attention in the past (Elliot *et al.,* 1972), there is currently an increasing evidence and wide acknowledgement that for elimination to be realized (Benelli and Beir, 2017), interventions that tackle residual transmission should be prioritized (Mwesiga *et al.,* 2017). In sub - Saharan Africa, Tanzania is a vivid example of the dual complexity in pushing malaria control further down. In southern and north western regions, Malaria has remained persistently high (11.7% to 24.7%) inspite of the wide coverage with LLINs, IRS and case management using Artmether-Lumephantrine (TMIS,2017). For example, in the Lindi region, the average regional malaria prevalence remains high at 11.7% (TMIS, 2017), but with great village variations. For example, in the Ruangwa District, Malaria prevalence rate of 17.7%, 54.3% and 85.7% were recorded from the villages of Likangara, Nandagara and Chienjere respectively (DHIS, 2016).

However, the main contributing factors for this persitence in malaria transmission despite widespread use of the current core vector control intervention measures are not well known. In addition, factors such as; unkown species which reduced behaviour succesptibility to indoor insecticidal intervention (Russell, *et al.,* 2011), and rapid expansion of insecticide resistance to malaria vectors (Nkya, *et al.,* 2013) could be playing an important role in malaria transmission. Furthermore, the emergence of antimalaria drug-resistant to strains of malaria parasites (WHO, 2019), insuficient levels of access and up take of lifesavings malaria tools preferably early diagnosis and treatment at village levels have been documented (Huho *et al.,* 2013). Substantial research evidences on this entomological factors is still limited (Govella *et al.,* 2013). Therefore, the major aim of the present study was to identify entomological drivers for persisting high malaria transmission despite high coverage with long lasting insecticides treated nets. The information obtained could be used as a benchmark for comprehensive approach of new tool and strategies to address residual transmission burden.

Methodology

Study area: The study was conducted in Ruangwa district, populated with 131,080 people. It's a flat land with hilly areas, the altitude is 313 - 549 meters above sea level. It has 160.1 hectares of 2,850 km² potential areas for irrigation. There's one rainy season (November – May), averaging 800 mm of rainfall per year. The daily temperature ranges from 24 to 35 degrees Celsius with very high humid air; a source of malaria prevalence rate. The three study villages (Likangara, Nandagara and Chienjere) have rural-urban and para-rural settings with low malaria prevalence of 17.7%), moderate 54.3% and high 85.7% respectively. (DMIS, 2016). The villages are located in low savannah area covered with grasses, bushes and scattered trees, narrow slow running streams with marginal vegetation and pad fields, shallow wells, bored wells and ponds which are seasonal breeding habitats. Many villagers are agriculturalists, few are petty traders. Numerous livelihood social - economic activities are conducted along main roads during nocturnal hours. These habits placing them at a greater risk of contracting malaria (Monroe *et al.,* 2019).

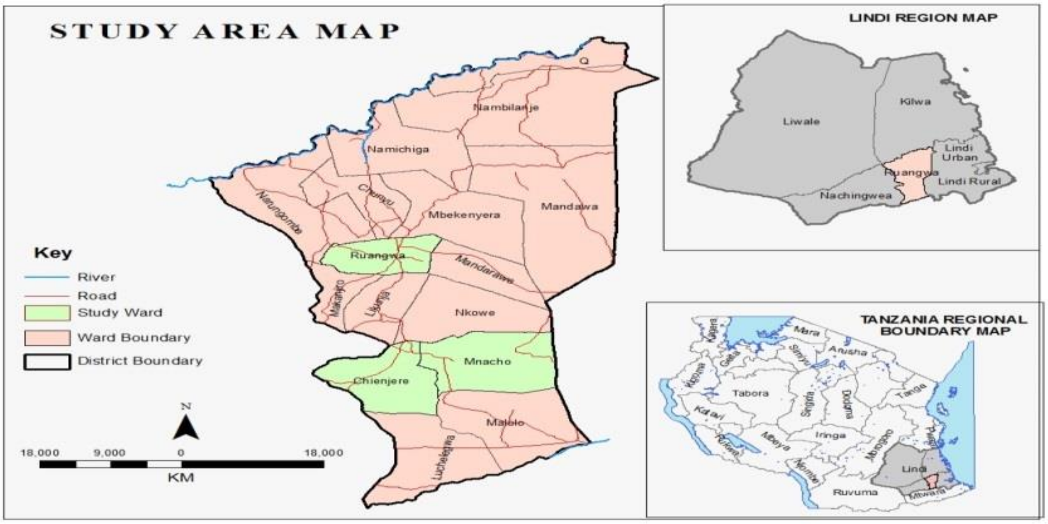

Figure 1: Maps showing the region, district and 3 villages where malaria vectors collected

Mosquito sampling, field processing and species identification; Mosquito collections were conducted in the three wards of the Ruangwa District, Lindi Region (Figure 1). Human landing catches (HLC) were used to collect host seeking mosquitoes (Service, 1993). Mosquito collections were conducted for three consecutive nights weekly between 1800hrs and 0700hrs from March, 2017 to July 2018. Mosquitoes were collected indoors and outdoors in 18 houses (NIMR, 2011) randomly selected houses situated at least fifty meters apart in the three villages (Killeen *et al.,* 2006). A team of two trained collectors per house (one sitting indoors and another sitting five meters outdoors collected host seeking mosquitoes as they landed on their exposed their legs). Mosquitoes coming to bite the collectors were detected using a flashlight and were aspirated and placed in paper cups (Mboera, 2005). Each morning all collected mosquitoes were killed in the field using diethyl ether. Thereafter female *An. gambiae* s.l. and *An. funestus,* were identified, morphologically counted and stored in labelled Eppendorf tubes with dry desiccant (silica gel) for later laboratory processing at Ifakara Health Research and Development Centre (IHRDC) (Gillies and De Mellion, 1968). Other *Aedes* and *Culex* species mosquitoes were scored and discarded.

Laboratory Processing of Mosquitoes; Polymerase chain reaction (PCR) technique was used to differentiate sibling species belonging to the different species complex group using Genomic DNA extracted from both *An. gambiae* s.l and *An. funestus* complexes adult mosquitoes' legs (Scott *et al.,* 1993). The legs of each individual adult mosquito were placed in 1.5 ml Eppendorf tube followed by addition of 20 μl of TE buffer, tris –EDTA. The specimens were then incubated at the temperature of 95°C for 15 minutes in a heating block. The tubes were vortexed for 2 minutes and then the DNA containing supernatant was separated by centrifuging at 12000 rpm at a room temperature for two minutes. After amplification 13 μl of PCR products were analyzed. The four positive controls for *An.gambiae s.l*, *An.funestus*, *An.arabiensis*, *An.quadriannulatus* and *An.merus* and the control for *An.funestus: An.rivulorum, An. vanedeen, An. parensis* and *An.leeson* were obtained from Ifakara and Maureen's insectary. DNA bands were revealed and photographed under ultraviolet light using Kodac. The presence of infection rates in infected *anophele*s mosquitoes species the infection rate in malaria vectors with *plasmodium falciparum* within collected mosquitoes were detected by Circumsporozoites protein (CPS ELISA) (Burkot *et al.,* 1984).

Statistical Analysis; Analysis was processed using Statistical package for the Social Science (SPSS), IBM Statistics version 20 (2018). The total *Anopheline* mosquitoes captured by human landing catches (HLC) on the same night was calculated by the numbers of *Anopheles* collected by species and the percentage divided in sub genus for each of these 3 villages (Likangara, Nandagara and Chienjere). These mosquitoes were tested and analysed by Chi-square test.

Results

The proportional abundance of mosquitoes captured in Ruangwa District

Abundance of mosquito species composition; A total of 2,532 female mosquitoes were collected between March 2017 and September 2018 in 18 houses from the study area. Out of these malaria vectors *An.gambiae* s.l constituted 26.35 (n=680), while *An.funestus* group comprised 0.31% (n=8) and *An.coustani* s.l 0.11% (n=3). In non-malaria vectors collected, *Culex* sp. was the dominant species with highest density of 73.2% (n=1,854), but *Aedes* sp. was composed a low density at 0.03% (n=1). The high density of mosquitoes was collected in Chienjere village 37% (n=932), followed by Likangara village 32% (n=820) and the least one was Nandagara village 31% (n=780). None of the *An. funestus* and *An.coustani* s.l was collected in Nandagara and Chienjere villages (Table 1) This implies that mosquitoes density varied from one species to another ($\chi 2$ = 80.82, df=3, p< 0.005). In addition, the detailed results of *Anopheles* mosquito's species are further discussed in the following section.

Table 1: Mosquito species composition captured in Ruangwa District

Table 4.1: The proportional abundance of mosquitoes captured in Ruangwa District

Village	***Culex sp.***		***An.gambiae s.l***		***An.funestus***		***An.coustani***		***Aedes sp.***		**Overall**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**N**	**%**
Likangara	438	24	373	55	6	100	3	100	1	100	**820**	**32**
Nandagara	745	40	35	5	0	0	0	0	0	0	**780**	**31**
Chienjere	671	36	261	40	0	0	0	0	0	0	**932**	**37**
Total	**1853**	**100**	**678**	**100**	**6**	**100**	**3**	**100**	**1**	**100**	**2532**	**100**

The composition of *Anopheles* species confirmed as malaria vectors by polymerase chain reaction (PCR) method; A total of 688 of *An.gambiae* s.l and *An.funestus* group were further subjected to PCR analysis for sibling species identification (Scott *et al.,* 1993). The PCR results indicated that (84.4%, n= 581) were confirmed as malaria vector species identified for the first time in the study area. In *An.gambiae* s.l two species of were identified, these are *An.gambiae* s.s 43 % (n=297) and *An.arabiensis* 40% (n=278), whereas in *An.funestus* group, only *An.funestus* s.s 1% (n=6) was also identified (Figure 2). Few unamplified mosquitoes constituted 16 % (n=107) of all *anophelines* collected. The DNA unamplified specimen may have been due to lack of appropriate primers in the PCR to identify migrant species. There was a considerable variation between *An.gambiae* sensu lato and *An.funestus* (χ^2=79.93, df =3, p<0.005).

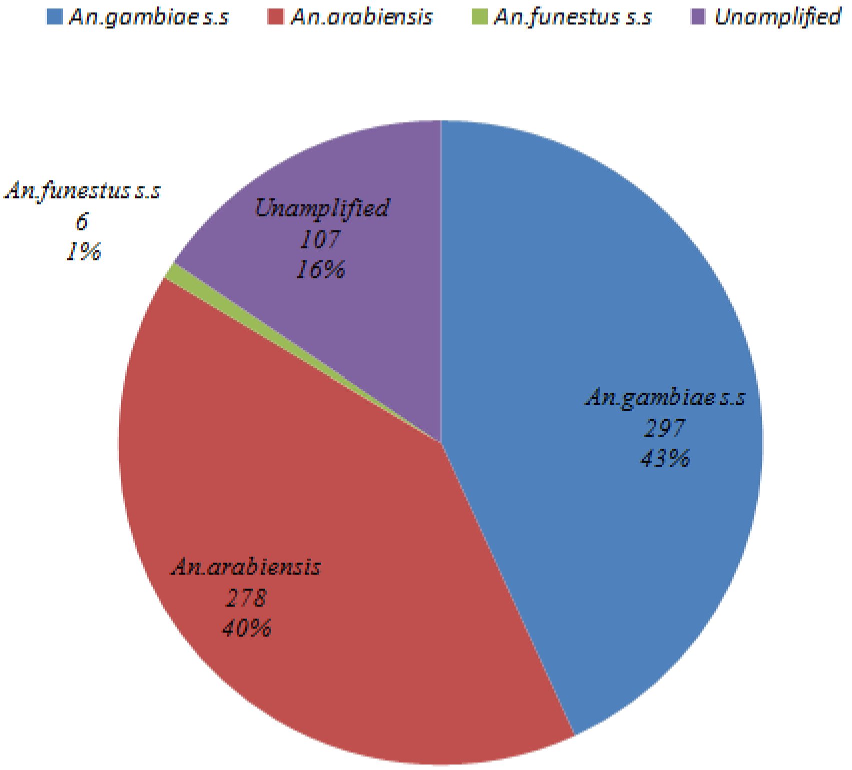

Figure 2: The proportional abundance of *Anopheles* mosquitoes captured in Ruangwa District

In addition, out of four species *An. gambiae* s.s was the most abundant to *An.arabiensis* in Likangara villages as compare to other villages of Nandagara and Chienjere and they constituted 167 (56.22%) of the total *An.gambiae* s.l speciated. Similarly, *An.arabiensis* was dominant species in

Chienjere village and constituted 147 (52.87%) of the total speciated *An. arabiensis*. Likewise, *An.funestus* s.s represented 6 (100%) of the total *An.funestus* speciated and was collected in Likangara village. None of the *An.funestus* s.s was found in Chienjere and Nandagara villages. There was a significant difference in mosquitoes collected between *An.gambiae* s.s, *An.arabiensis* and *An. funestus* in the study area (χ^2=79.93, df =3, p<0.005).

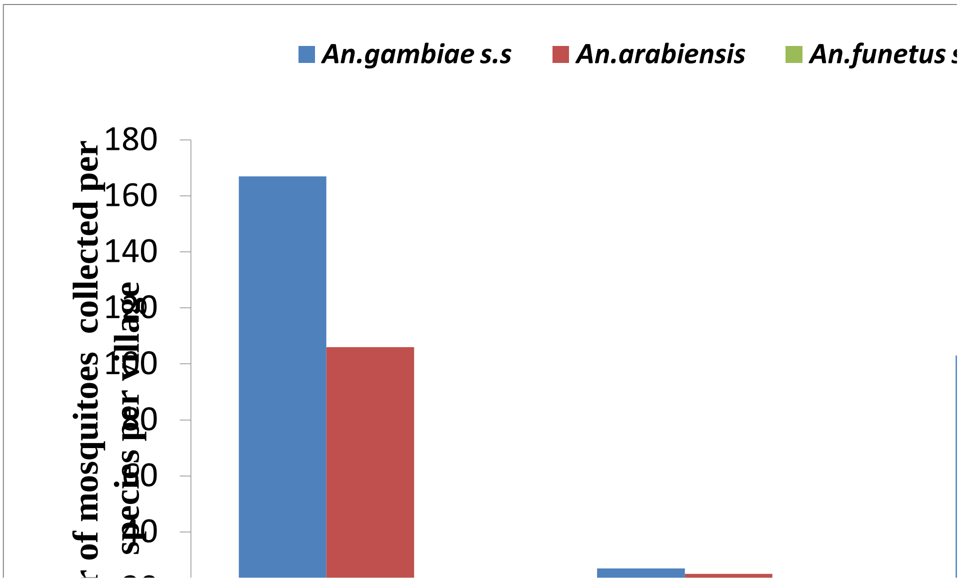

Figure 3: Variation of *Anopheles* mosquito population collected in the three villages of Ruangwa District

A study of seasonal population dynamics of malaria vectors detected variation between species collected during the rainy season (95.17%,n=553) and in the dry season (4.81%,n=28). While the population of *An.funestus* decreased during the rainy season (0.34%, n=2), it increased in the dry season (0.66%, n=4). Chienjere village had high mosquito density of (52.34%,n=298) followed by Likangara(n=203) and (9.12%, n=52) Nandagara village in both seasons. Within species, a large number of mosquitoes was *An. gambiae* s.s was observed to be high at 94.27%, n=280) in the rainy compared to dry season (5.73%,n=17). While in *An.arabiensis* (97.48%, n=271) was collected in the rainy season and (2.52%, n=7) in the dry season and *An.funestus* s.s consisted of (33.3%, n=2) in the rainy season and (66.6%, n=4) in the dry season (Table 3).

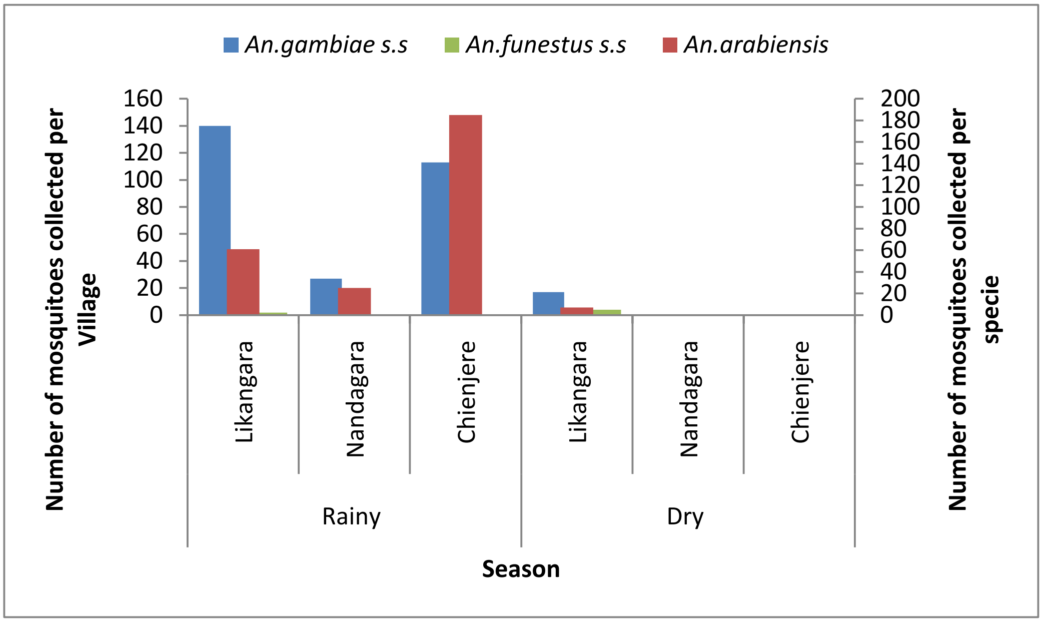

Figure 4: Seasonal variation of malaria vectors between species by villages and species

Discussion

Species Composition of Malaria Vectors available in Ruangwa District

It is important to note that, this is the first report on speciation of *Anopheles* mosquito species in Ruangwa, Lindi region. Although these malaria vectors have been reported in other regions of Tanzania, there was no information on species composition available in Ruangwa district. The results show presence of two broad efficient malaria vectors species of *An. gambiae* s.s and *An. arabiensis* from *An.gambiae* complex. Secondary malaria vector species are; *An .funestus* s.s from *An. funestus* group and *An. coustani*.The first two species are responsible for maintaining high prevalence of malaria even in the absence of other vectors (Colluzzi, 1984; PMI, 2018).

Using the human landing catches Service (1993), this study ought to answer the current baseline data on composition of malaria vector species available in Ruangwa district. In the present study, based on the results an affirmative answer has been found, the composition of malaria vector species available in Ruangwa district entails new four potential malaria vector species, namely: *An.gambiae s.s and. An. arabiensis, An.funestus s.s* and *An.coustan*i. The first two spiciest are responsible for maintaining high prevalence of malaria even in the absence of other vectors (Colluzzi, 1984 and PMI, 2018). This is an important step towards the control of malaria vector populations which influence ongoing residual malaria transmission in Ruangwa district (Mwanziva,2011; Stevenson *et al.,* 2016 and; Degefa *et al*.,2017).

The significance of this discovery is that this information forms a benchmark which will be integrated in a national map of dominant malaria vector species as well as assisting in the planning and implementation of malaria prevention interventions. According to (Coetzee, 2000; Sinka *et al.,2012*), this claim is premised on the fact that identifying species leads to associate knowledge of that species which in turn suggests effective approaches for malaria control interventions. Coetzee, (2004) observed that *An. Quadriannulatus* a non malaria was more susceptible to organochlorine (Benzene hexachloride), sprayed indoors than malaria vectors *An.arabiensis* that were resistant during the malaria epidemic that occurred in 1977 in Zimbabwe. This led to a policy change to shift to the use of DDT that gave greater impact in prevalence reduction. In addition, in Cameroon (Tabue,2014) identified *An. ziemanni* as a main malaria vector compared to other *Anopheles* vectors such as *An.gambiae* s.s and *An. christy, An.nil, An.maculipalips, An.implexus, An.funestus, An.pharoesnis* and *An tenebrosus*. This led to a change in vector control strategies. Therefore, the result of the present study concurs with Erlank (2018), who recorded *An.* a*rabiensis* and *An.gambiae* as a main vectors needed to improve malaia control efforts and control strategies for malaria elimination.

There was generally seasonal preference by vector species which subsequently affect their distribution in the study area. The high density of mosquitoes was collected during rainy season 95.18% and few (4.56%) figure 4. This result coincides with ones reported in other studies done by (Kigadye *et al.,* 2010; Kulkarni, *et al.,* 2010; Kabbale *et al.,* 2013) in other parts of Tanzania and Uganda, that rainfall favours the high abundance of *An. gambiae* populations and doubles the increase of malaria infections. In the present study, *An. funestus* is the most important dry season malaria vector as compared with a rainy season and can be able to maintain the population of the mosquitoes year-round (Figure 4). This result is also consistent with one obtained by (Minakawa *et al.,* 2005; Lwetoijera,2014) that *An. funestus* were more abundant in the dry season as compared to rainy season in Kenya and Kilosa Tanzania. Other non-malaria vectors of medical importance collected in the study area *Culex* species; In the present study, two genera of *Culex.sp* and *Aedes sp*. non- malaria vectors were collected from three village of Ruangwa District. *Culex sp*. was the dominant species with highest number (72.87%) among all mosquitoes collected (Table 1). The result concurs to studies done at Niete, Cameroon (Bigoga *et al.,* 2012) which also found the dominance of *Culex* sp. over *Anopheles* sp; (Emidi *et al.,* 2017). The higher density of *Culex* sp. could be attributed to their preference to breed in dirty water which is available throughout the year. The sampled houses in the villages of Likangara, Nandagara and Chienjere are located near wet pit latrines and uncontrolled waste. These findings are also similar to the findings of earlier study conducted by (Nwoke *et al,* 1993; Rwegoshora *et al.,* 2005), they found that the abundance of mosquito was homogenous in polluted water. Therefore, C*ulex* sp. the vector of *W. bancrofti* should be of great epidemiological concern to policy makers and researchers since it may pose a great threat to humans in future. This is because *W. bancrofti* transmission is still ongoing in the neighbouring region of Pwani; in Mafia Island despite long term use of community mass administration drugs for about 15 years and twelve rounds in Rufiji district (Jones *et al.,* 2015; Jones *et al.,* 2018).

Aedes species; *Aedes* was the least species having a lower number of mosquitoes among collected species (0.51%, 1). The low number was probably a consequence of ecosystem and feeding preferences. *Aedes* species is a small container available with water, year-round breeder and do not depend on rainfall availability Huber *et al.,* (2002). These species breed in any storage container. In the present study, there was a low close to zero density of *Aedes* sp. during March and April (the months of heavy rainfall in rainy seasons). This result is the same as reported by (Coulibaly *et al.,* 2010; Mohan *et al.,* 2014) in their study on distribution and seasonal variation of *Aedes aegypti* in Abidijan, Cote D'ivore, Kolkata, India and Argentina. They reported a significant decrease in *Aedes aegypti* mosquito abundance during the period of high rainfall, the rains overfilled storage containers and female mosquitoes lacked space for eggs deposition and this could probably the reason for low density of *Aedes* mosquito collected in the study area (Powell and Tabachnick, 2013). However, the findings of the present study are contrary to the findings reported by (Emid *et al.,* 2017). They reported that, during their study, in Muheza Tanzania, mosquitoes of *Aedes* species were abundant during the dry season compared to the rainy season. Conversely, the low density of *Aedes* species collected in Ruangwa district warrants further investigation.

Therefore, identification of the local malaria vector responsible for high malaria prevalence in a study area is an important step in acquisition the information on species composition available, their distribution and role in the ongoing residual malaria transmission (Erlank *et al.,* 2018). This observation would guide in the planning and and implementation of effective vector control strategies.

Conclusion and Recommendation

Conclusions

The results have shown that: *An. gambiae* and *An. arabiensis* are important local malaria vector in Ruangwa district and *An. coustani* s.l and *An. funestus* are less important vectors, but an incidental *An.coustani* has epidemiological concern since it is an important malaria vector in neighbouring countries of Kenya and Zambia along with other African countries like Nigeria and South Africa. The abundance of *Anopheles* mosquitoes are high in rainy season as compared to the dry season. *Culex* species are also found in high density in Ruangwa district. These mosquito species are considered as one of the potential vectors causing Lymphatic filariasis and there is evidence of the presence of microfilaria in human populations in the nearby coastal regions. Therefore, mapping of these vectors in national map of dominant species as identified for the first in Lindi region, planning and effective control and elimination strategies of malaria vector and *Culex* species in the study area would eliminate the ongoing malaria transmission and the presence of microfilaria risks which are ongoing in the neighbouring coastal regions of Tanzania.

References

Benelli, G. and Beir, J.C. (2017). Current vector control challenges in the fight against malaria. *Acta Tropica;* 10 (174), 91-96.

Bigoga, J. D., Nanfack, F. M., Awono-Ambene, P. H., Patchoké, S., Atangana, J., Otia, V. S. and Leke, R. G. (2012). Seasonal prevalence of malaria vectors and entomological inoculation rates in the rubber cultivated area of Niete, South Region of Cameroon. *Parasites and vectors* 5(197), 1186.

Burkot, T.R., Williams, J.L. and Schneider, I. (1984). Identification of Plasmodium falciparum infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. 33 (5), 783-788

Coetzee M, Craig M, le Sueur D. (2000). Distribution of African malaria mosquitoes belonging to the Anopheles gambiae complex. Parasitol Today., 16: 74-77.

Coetzee, M. (2004) Distribution of the African malaria vectors of the *Anopheles gambiae* complex. Am. J. Trop. Med. Hyg. 70**,** 103-104.

Coulibaly, G. N., Adja, M. A., Kondou, G. B., Konan, L. Y., Diallo, M.,, and Goran, E. K. (2010). Distibution and Seasonal Variation of *Aedes aegypti* in the health district of Abidjan (Cote D' Ivore) *European Journal of Scientific Research*, 40, 522-530.

Degefa, T., Yewhalaw, D., Zhou, G., Lee, M.C., Atieli, H., Githeko, A. K., and Yan., G. (2017). Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission. *Malaria Journal* 16 (443).

DHIS (2017). Tanzania National Health Portal Retrieved on 4th Aug, 2018 https://hmisportal.moh.go.tz/hmisportal/#/pages/home/programs/nmcp/malaria_hiv_tb

DHIS2 (2019). Malaria Surveillance B 2015 ulletin-2018, National Malaria Control Programme, Dodoma, Tanzania; Issue no 7:2015-2018 National Health Portal Retrieved on 4th February, 2019 https://hmisportal.moh.go.tz/hmis portal/#/pages/home/programs/nmcp/malaria_

DHIS.(2017).Tanzania National Health Portal Retrieved on 4th Aug, 2018 https://hmisportal.moh.go.tz/hmisportal/#/pages/home/programs/nmcp/malaria_hiv_tb

Dida, G.O., Anyona, D.N., Abuom, P.O, Akoko, D., Adoka S.O and Owour, P.O. (2018). Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. *Infect Dis Poverty* **7,** 2

Emidi B., Kisinza W.N., Stanley G., and Mosha F.W. (2017). Seasonal Variation of Journal of Public Health and Epidemiology 3(1), 1040

Erlank, E., Koekemoer, L.L. and Coetzee, M. (2018) The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. *Malar J 17***,** *43.*

Gatton. M.L., Chitnis. N., Churcher, T., Donnelly, M.J., Ghani, A.C., Godfray, H.C.J. and Ranson, H. (2013). The importance of mosquito behavioral adaptation to malaria control in Africa. *Evolution*, (67), 218-1230.

Gillies M.T. and DeMeillon B. (1968). The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). Johannesburg: *South African Institute for Medical* Johannesbug South Africa. Research. Publication No.54.

Gillies, M.T. and Coetzee, M. (1987). A supplement to the Anophelinae of Africa South of the Sahara. *Publication of South African Institute for Medical Research,* Johannes South Africa 55: 1-143.

Huber, K., Luu, L. L., Tran, H. H., Khanh, T., Rodhain, F., and Failloux, A. B. (2002) .Temporal genetic variation in *Aedes aegypti* populations in Ho Chi Minh City, Vietnam. *Nature publishing group*, 89, 7-14.

Huho, B., Briët, O., Seyoum, A., Sikaala, C., Bayoh, N. and Gimnig, J. (2013). Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa. *International Journal of Epidemiology*, 42: 235–47.

Jones C.,Ngasala B., Derua Y.A., Tarimo D., Reimer L., Bockarie M., and Malecela M.N.(2018). *Lymphatic filariasis* transmission in Rufiji District, south-eastern Tanzania: infection status of the human population and mosquito vectors after twelve rounds of mass drug administration. *Parasites and Vectors.* 13; (1):588.

Jones C, Tarimo, D.S, and Malecela, M.N. (2015). Evidence of continued transmission of *Wuchereria bancrofti* and associated factors despite nine rounds of ivermectin and albendazole mass drug administration in Rufiji district, Tanzania. *Tanzania Journal Health Research***.** 17:2.

Kabbale, F.G, Akol, A.M., Kaddu, J.B. and Onapa, A.W. (2013). Biting patterns and seasonality of *Anopheles gambiae* sensu lato and *Anopheles funestus* mosquitoes in Kamuli District, Uganda. *Parasites and Vectors*, 6.340.

Kaindoa, E. W., Halfan S. Ngowo, Alex J. Limwagu, Magellan Tchouakui, Emmanuel Hape and Fredros O. Okumu. (2019). Swarms of the malaria vector Anopheles funestus in Tanzania. *Malaria Journal* 18.29.

Kigadye E.S.P., Nkwengulila G, Magesa S.M., and Abdulla, S.(2010). Diversity Spatial and temporal abundance of *Anopheles gambiae* complex in the Rufiji River basin, south-eastern Tanzania. *Tanzania Journal of Health Research*, 12: 68–72

Killeen G.F., Kihonda J, Lyimo E., Oketch F.R., Kotas M.E., and Mathenge E. (2006). Quantifying behavioural Interactions between humans and mosquitoes: Evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. BMC Infect Dis 2006; 6:161.

Killeen, G.F. (2014). Characterizing, Controlling and Eliminating Residual Malaria Transmission. *Malaria Journal* 13: 330.

Kisinza, W, N., Nkya, T.E Bilali, K., Overgaard, H. J., Massue, D.J., Mageni, Z.,. and Magesa S. (2017). Multiple insecticide resistance in *Anopheles* *gambiae* from Tanzania: a major concern for malaria vector control. *Malaria Journal* 16: 439 .

Kulkarni, M. A., Desrochers, R. E., and Kerr, J. T. (2010). High resolution niche models of malaria vectors in northern Tanzania: a new capacity to predict malaria risk?. *PloS one*, 5(2), e9396.

Lwetoijera, D. W., Harris, C., Kiware, S. S., Dongus, S., Devine, G. J., McCall P. J. and Majambere, S. (2014). Increasing role of Anopheles funestus and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. Malaria Journal 13(1): 331.

Main. B.J, Lee, Y. Ferguson, H.M. Kreppel, K.S., Kihonda, A. and Govella, N.J. (2016). The genetic basis of host preference and resting behavior in the major African malaria vector, *Anopheles arabiensis*. *PLoS Genetics*, 12(9): e1006303.

Mboera L.E.G. (2005). Sampling techniques for adult Afrotropical malaria vectors and their reliability in the estimation of entomological inoculation rates. *Tanzania Health Res Bull* 7: 117-124.

Mboera, L.E.G., Bwana V. M., Rumisha S. F., Stanley, G., Tungu P. and Malima C. (2015) Spatial abundance and human biting rate of Anopheles arabiensis and Anopheles funestus in savannah and rice agro-ecosystems of Central Tanzania ,Geospatial Health 10:322

Minakawa N., Munga S., Atieli F., Mushinzimana E., Zhou G, Githeko A.K and Yan G. (2005). Spatial distribution of anopheline larval habitats in Western Kenyan highlands: Effects of land cover types and topography. *Am J Trop Med Hyg*; 73:157–165.

Mohan S., Banerjee S., Mohanty S.P, Saha G.K, and Aditya G. (2014). Assessment of pupal productivity of *Aedes* and co-occurring mosquitoes in Kolkata, India. *Southeast Asian Pacific Journal of Tropical Medicine Public Health*. 45: 1279–1291.

Mwesigwa J., Achan J., Tanna, G.L, Affara, M., Jawara M., and Worwui A. (2017). Residual malaria transmission dynamics varies across The Gambia despite high coverage of control interventions. *PLoS ONE* 12(11).

Mwanziva, C. E., Kitau, J., Tungu, P. K., Mweya, C. N. Mkali, H, Ndege, and Mosha, F. W. (2011). Transmission intensity and malaria vector population structure in Magugu, Babati district in Northern Tanzania. *Tanzania Journal of Health Research* 13(1), 68 - 78.

Nkya T.E., Akhuyril I., Kisinza W., and David J.P. (2013). Impact of environmental mosquito response to pyrethroids insecticides facts, evidences and prospects, *Insect Biochemical Molecular Biology*, 43: 407-416.

Nwoke, B., Nduka, F. O., Okereke., O. M., and Ehighibe, O. C. (1993). Sustainable urban development and human health: septic tank as a major breeding habitat of mosquito vectors of human diseases in south-eastern Nigeria, *Applied Parasitology*, 34 (1), 1–10.

PMI (2015). President Malaria Initiatives –Tanzania Malaria Operation plan financial year 2014. Retreived on 28th Dec, 2016 from https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy-15/fy-2015-tanzania-malaria-operational-plan.pdf .

PMI (2018). President Malaria Initiatives -Tanzania. Malaria Operation plan financial year 2017. Retrieved on 05th Dec, 2018 from https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy-2018/fy-2018-tanzania-malaria-operational-plan

Powell R.J and Tabachnick W.J (2013). History of domestication and spread of Aedes aegypti- review.108 (suppuliment1). Retrieved on 12th Jul.2016 from https://www.Researchgate .net/ publication/ 259960177. History of domestication and spread of Aedes Aegypti Aedes Review.

Protopopoff N., Matowo J., Malima R., Kavishe R., Kaaya R., Wright, A. (2013). High level of resistance in the mosquito Anopheles gambiae to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania.*Malaria Journal*,12:149.

RDSS. (2016). Ruangwa Demographic Surveillance System, Ruangwa Lindi.

Rwegoshora, R., Pedersen, E., Mukoko, D., Meyrowitsch D.W., Masese N, and Malecela-Lazaro, M, (2005). Bancroftian filariasis: patterns of vector abundance and transmission in two East African communities with different levels of endemicity. *Ann Trop Med Parasitol*., 99: 253–65.

Scott, J.A., Brodgon, W.G. and Collins, F.H. (1993). Identification of single specimens of *Anopheles gambiae* complex by Polymerase Chain Reaction. *Am J Trop Med Hyg* 49, 520-529.

Service, M. (1993) *Mosquito Ecology: Field Sampling Methods*. Applied Science Publishers, London, 583pp.

Sinka M.E., Bangs M.J., Manguin S, Rubio-Palis Y., Chareonviriyaphap T.,and Hay S.I. (2012). A Global map of dominant malaria vectors. *Parasites and Vectors* 5: 69.

Staedke S. G., E. W. Nottingham, J. Cox, M. R. Kamya, P. J. Rosenthal, and G. Dorsey (2003). Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children,*The American Journal of Tropical Medicine and Hygiene*, 69(3), 244–246.

Ssemppira, J., Namubus,B., Kisa, J., Agba B., Makumbi, F., andVounatsou, P. (2017). The contribution of malaria control intervention on spatial-temporal changes of parasitaemia risk in Uganda during 2009-2014. *Parasites and Vectors*, 10,450.

Stevenson, J. C., Simubali, L., Mbambara, S., Musonda, M., Mweetwa, S., Mudenda, T., Pringle JC, Jones CM, and Norris, D. E. (2016). Detection of *Plasmodium falciparum* Infection in Anopheles squamosus (Diptera: Culicidae) in an Area Targeted for Malaria Elimination, Southern Zambia. *Journal of medical entomology*, 53(6), 1482–1487.

Tabue, R. N., Nem, T., Atangana, J., Bigoga, J. D., Patchoke, S., Tchouine, F., Fodjo B.Y., Leke R.G., Fondjo, E. (2014). *Anopheles ziemanni* a locally important malaria vector in Ndop health district, north west region of Cameroon. *Parasites and Vectors*, 7**,** 262.

The National Institute for Medical Research (2011). Standard operating procedures for surveillance of disease vectord within the context of intergrated disease surveillance and intergrated vector management in Tanzania; 1(3),10-16.

Tanzania HIV/AIDS and Malaria Indicator Survey report (2012). Dar Es Salaam, Tanzania. https://dhsprogram.com/pubs/pdf/AIS11/AIS11.pdf

Tanzania Malaria Indicator Survey (2017). Tanzania malaria indicator survey 2017, Dar es Salaam, Tanzania. Retrieved on 21st September, 2017 from htpps://dhdprogrsm.com pdf

Wiebe, A., Longbottom J., Gleave, K., Shearer, F.M., Sinka, M.E., and Massey, N.C. (2017). Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. *Malaria Journal*; 16 (1): 85

World Health Organization (2018). World Malaria Report World Health Organization, Geneva. https:// www.who.int/malaria/ publications/ world-malaria-report-2018/en/

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Author's contributions

Clement Godfrida was the principal investigator of the study and led collection of data, designed the sampling technique, conducted data analysis, and interpreted results. Prof. Emmanuel S.Kigadye and Dr. Nicodem J. Govella provided technical support guidance. Ifakara Health Institute laboratory Staff provided sibling species analysis protocol. All authors read and the Open University approve the manuscript.

Conflict of interest: The authors declare no conflict of interest

Ethical clearance: This received an ethical approval from the Medical Research Coordination Committee of the National Institute of Medical Research in Tanzania Reference no NIMRI/HQ/R.8a/Vol. XI /3232. Collectors were employed voluntarily with oral and written consent and advised to withdraw to from the study at any time. Before the study all collectors were screened every week for malaria and were given chemoprophylaxis malarone® without any payment. Anybody found positive was discontinued, fortunately no collector contracted malaria during the experimentation process. Permission to enter households was obtained from household heads after explaining the objective and benefit of the study.