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Common Types of *Anopheles Gambiae* Breeding Habitats in North Western Nigeria

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Abstract: Malaria constitutes a major public health concern in Nigeria, where it is exacerbated by very high vectorial density due to favourable breeding conditions. This study investigates whether the links between physicochemical characteristics of An. gambiae breeding habitats and their productivity (larval density) can be used to finger print and define mosquito breeding habitats based on three most common human related activities (residential, agricultural and petrochemical) in Northern Nigeria. The characteristics and levels of 13 physicochemical environmental factors and breeding sites productivity were determined in three common mosquito breeding habitats (designated as study zones). Biological oxygen demand (BOD), total dissolved solids (TDS) and all the six chemical factors examined differed significantly ($p<0.05$) across the three study zones. Specifically sulphates, phosphates, nitrites and nitrates levels were significantly higher in the agricultural areas, while carbon content and oil and grease were significantly higher in the petrochemical laden breeding ecologies. An. gambiae larval density was significantly higher in domestic environments compared to the other two studied zones and correlated positively with BOD and transparency ($p<0.05$). Contrarily, the larval density correlated negatively with pH, temperature and all the chemical factors ($p<0.05$). An. gambiae breeding sites productivity was significantly affected by the levels and characteristics of some physicochemical environmental factors, which also weightily impacted to segregate and distinctly define the major human related activities taking place within and/or around the sites. This could pose a serious but different challenge to environmental management and insecticides based approaches to malaria vector control in Nigeria.

Keywords: Anopheles gambiae, breeding habitats, larval density, North Western Nigeria, Mosquito

INTRODUCTION

According to the World Health Organisation (WHO), about half of the world population (3.3 billion people) is at risk of malaria. Although the past several years have witnessed tremendous increase in control measures, malaria still remains the number one killer disease especially in Sub-Saharan Africa. More than 216 million cases were reported in 2010 alone, with over 660, 000 deaths recorded (WHO, 2013). In Nigerian, the entire population of over 170 million is at risk of malaria which is responsible for about 60% and 30% of outpatients' visits and hospitalisations respectively (PMI, 2013). Insecticide-based measures (insecticide-treated mosquito nets, ITNs or long lasting insecticide nets, ILLINs and indoor residual spraying, IRS) are widely used to control malaria vector. These measures are currently inadequate (Okorie *et al*., 2011) and not sufficient to halt malaria transmission and would likely contribute to the eventual emergence of insecticide resistant mosquitoes (Ranson *et al*., 2009; Okia *et al*., 2013). Currently, researchers have been exploring several alternative avenues of controlling malaria, and one particular approach that appears to be gaining attention is an environmental management strategy that aims to reduce adult vector population by targeting their aquatic immature stages (i.e. mosquito eggs, larvae and pupae). This strategy is becoming increasingly important in many countries especially in sub-Saharan African and involves different species of mosquitoes including those that transmit malaria. The strategy depends on the use of various larvicidal techniques and environmental management practices aimed at reducing larval density and therefore minimizing or reducing vector abundance (Gu & Novak, 2005).

However, since mosquito breeding sites are found in various environments ranging from farmlands to sites of industrial activities, deliberate human intervention at controlling mosquito populations may not be the only contribution to the factors affecting larval growth and development. For

instance, some of the most active breeding ecologies for mosquitoes are located around farmlands where various agro-allied chemicals such as fertilizers, pesticides and herbicides, are applied to enrich soil and control agricultural pests and diseases (Antonio-Nkondjio *et al*., 2012; Afrane *et al*., 2012). Active mosquito breeding sites have also been reported in areas polluted by industrial effluents, rotting vegetation, human faeces, cow urine, as well as oil and grease mostly in urban centres (Awolola *et al*., 2007). In contrast, mosquito larvae have been found breeding in clearer, cleaner and apparently less contaminated surroundings usually around human habitation. The factors governing the choice of water bodies by female mosquitoes to lay their eggs for breeding is still poorly understood and the knowledge of the mechanisms behind habitat selection by most species of mosquitoes is still at its infancy (Fillinger *et al*., 2004). What is clear though, is that mosquito breeding sites are located in a variety of environments; contaminated and uncontaminated, and larval development and survival may be attributed to degree of contamination, possible priming and selectivity for chemical tolerance and cross extended insecticide resistance (Yadouleton *et al*., 2011; Antonio-Nkondjio *et al*., 2012).

Several previous studies (Animut *et al*., 2012; Imbahale *et al*., 2011; Mala *et al*., 2011) have established the impact of several ecogeographical, topographical, agricultural, and other environmental indices on Anopheles larval diversity, abundance, and dynamics, as well as breeding sites productivity. However, few published data are available on the physical and chemical nature of *An. gambiae* breeding sites and its impact on larval behaviour, development, and survival. Also, there is dearth of base-line data regarding mosquito insecticide resistance and it's monitoring in Nigeria, as well as data regarding environmental monitoring and control of mosquito breeding sites. Importantly there are hardly any such relevant data covering the north western part of Nigeria since the Garki Project of 1960-70s (Okorie *et a.,l* 2011), despite the massive and accelerated population growth, urbanisation, agricultural and industrial expansions recorded in this region within the last four decades. This is the most populous geopolitical zone in Nigeria with a malaria prevalence of close to 50% (PMI, 2013). This present study reports the impact of the assessed presence, levels and characteristics of some physicochemical environmental factors on *An. gambiae* larval density in the most common types of breeding habitats based on human related activities within and around. The results weightily segregated to discriminate and define the three most common mosquito breeding habitats in Nigeria.

Methodology

Study Sites and Zones

The study was conducted across three different breeding ecologies designated as study zones A, B & C. These zones were differentiated by the type of human related activities taking place around the mosquito breeding sites i.e. A, intensive agricultural areas; B, residential areas; and C, areas where petrochemical products are sold, processed, used and discharged. A total of three sites in study zone A, four in zone B and three in zone C were sampled across the Nigerian states of Kano and Jigawa. Kano is situated in the northwest and has a four-season climate with a typical temperature range of 11-44°C and yearly rainfall of 1000mm. Jigawa is also situated in the northwest, and is characterised by a Sahel savannah climate with a typical temperature range of 10-42°C and a yearly rainfall of less than 800mm (John, 2007; NIMET, 2012).

Larval sampling and determination of larval density

Sampling of mosquito larvae from breeding sites identified in each of the three study zones was conducted at least once a week throughout the field study period (June-September, 2011). Larval collections were made using 350 ml standard mosquito dipper (WHO, 1975). Several dips were made depending on the size of the breeding habitat. Anopheles larvae in each dip was estimated by manual counting and overall larval densities were determined by direct measurement and expressed as number of larvae per litre of breeding water.

Species Identification

An. gambiae intraspecie identification was carried out by the standard PCR procedure described by Scott (Scott *et al*., 1993); courtesy of the Nigerian Institute of Medical Research, Yaba, Lagos-Nigeria.

Water chemistry analysis

Conductivity, pH, temperature, and total dissolved solids were measured using COMBO PH/EC/TDS/Temperature metre (HANNA Instruments, United States). Transparency (turbidity) was determined using a secchi disc. Dissolved oxygen (DO) and biological oxygen demand (BOD) were determined using a DO meter (Hach Lange, Colorado-United States) as described by Maiti (2004). Nitrate (No_3^-), Nitrite (NO_2^-), Phosphate (PO_3^{2-}), and Sulphate (SO_4^2) ions concentrations were determined by the sulphanilamide-N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride)

colorimetric, phenol disulphonic acid, stannous-chloride and turbidimetric methods, respectively (Maiti, 2004). Carbon content (total organic carbon) was determined using the Lange TOC cuvette-test (Salford United Kingdom). Levels of oil and grease were determined by the liquid-liquid extraction method described by Maiti (2004). Analytical grade chemicals and reagents used were from Sigma-Aldrich (United Kingdom) and BDH chemicals (VWR International Ltd. United Kingdom) unless otherwise indicated.

Data Analysis

Multivariate regression and factor analysis were employed to deduce correlations and relationships between the environmental factors and *An. gambiae* larval density. All the analyses were carried out with SPSS (SPSS Inc. SAS Institute) version 20.

Results

Specie identification

The Anopheles mosquitoes in this study were identified down to the molecular level as belonging to the members of the *An. gambiae sensu stricto*.

Relationship between levels of physicochemical environmental factors and larval density

In order to assess the thriving *An. gambiae* larval population (as a measure of breeding site productivity) and to evaluate the impact of physicochemical factors on larval survivorship, the levels of the physicochemical environmental factors and larval density were determined (Tables 1, 2 & 3), on a weekly basis and over the rainy season of 2011, from selected breeding sites and their mean distribution across the three study zones (A, B and C) was statistically evaluated. Then, relationships and correlations between the levels of the physicochemical environmental factors and larval density were examined within and across the three different breeding ecologies or zones.

Mean distribution of *An. gambiae* larval density

The results of one way ANOVA shows that there was a very highly significant difference (p=0.000) in mean larval distribution of *An. gambiae* across the three study zones with zone B (residential/domestic) having the highest larval mean density of 75/L The sampling sites within the intensive agricultural zone A were 2nd to those in zone B with a mean larval density of 29/L, which was about 38% of the larval productivity levels within zone B. The sites within the petrochemical laden breeding zone C had the lowest larval densities whose mean was about 1.9 and 5-fold lower than those observed for zones A and B, respectively. The results of the Bonferoni Post-hoc test carried out to examine the pairwise mean distribution of larval density across the three study zones showed that there were significant differences (p=0.000) in mean larval distribution between zone A & B; B & C; and A & C.

Table 1: Shows the values (mean ± SD) of the physico-chemical parameters and Larval density from sampling sites located in extensive agricultural areas. (Zone A)

	Sites of sampling		
Parameters	**Site 1**	**Site 2**	**Site 3**
pH	7.17±0.058	7.27±0.058	8.15±0.353
Temperature(^{o}C)	35.90±0.020	34.80±0.010	36.33±0.090
Transparency(cm)	3.50±.0.000	2.00±0.000	2.10±0.000
Conductivity(UmhoS/cm)	358.00±2.648	365.30±0.577	244.33±8.020
Dissolved Oxygen(mg/L)	2.33±0.115	1.83±0.057	1.63±0.058
Biological Oxygen Demand(mg/L)	1.73±0.115	1.23±0.058	1.13±0.057
Total Dissolved Solids(mg/L)	57.85±0.354	61.00±0.283	55.80±0.566
Sulphate ions(mg/L)	4.65±0.071	5.27±0.115	6.26±0.115
Phosphate ions(mg/L)	7.57±0.115	5.37±0.152	8.23±0.058
Nitrite ions(mg/L)	5.73±0.153	6.13±0.115	7.47±0.058
Nitrate ions(mg/L)	8.17±0.057	6.00±0.000	8.90±0.015
Carbon Content(mg/L)	1.59±0.055	2.00±0.000	2.85±0.071
Oil & Grease(mg/L)	ND	ND	ND
An. gambiae Larval Density (per litre)	40.00/L	25.71/L	20.00/L

Table 2: Shows the values (mean ± SD) of the physico-chemical parameters and Larval density from sampling sites located in domestic/residential areas (Zone B)

	Sites of sampling			
Parameters	**Site 1**	**Site 2**	**Site 3**	**Site 4**
pH	6.95±0.071	7.35±0.071	7.30±0.141	7.15±0.132
Temperature(^{o}C)	26.90±0.283	30.60±0.566	36.75±0.777	39.85±0.333
Transparency(cm)	1.90±0.000	1.20±0.000	6.50±0.000	1.30±0.000
Conductivity(UmhoS/cm)	227±1.414	393±7.071	367±12.730	395±2.828
Dissolved Oxygen(mg/L)	2.05±0.070	2.65±0.212	2.80±0.000	2.85±0.212
Biological Oxygen Demand(mg/L)	1.05±0.070	1.20±0.141	1.50±0.000	1.75±0.070
Total Dissolved Solids(mg/L)	17.85±0.212	22.00±1.414	13.00±0.283	27.35±0.212
Sulphate ions(mg/L)	1.11±0.177	2.32±0.043	2.03±0.084	1.93±0.092
Phosphate ions(mg/L)	1.04±0.078	1.43±0.035	1.46±0.098	2.04±0.078
Nitrite ions(mg/L)	1.42±0.092	1.90±0.098	2.67±0.134	1.52±0.042
Nitrate ions(mg/L)	1.70±0.035	2.37±0.035	2.52±0.042	2.03±0.099
Carbon Content(mg/L)	1.05±0.057	2.03±0.084	0.99±0.134	0.80±0.120
Oil & Grease(mg/L)	ND	ND	ND	ND
An. gambiae Larval Density (Per Litre)	100/L	40/L	100/L	60/L

Table 3: Shows the values (mean ± SD) of the physico-chemical parameters and Larval density from sampling sites located in areas where petrochemicals/hydrocarbons are processes, used and/or discarded (Zone C)

	Sites of sampling		
Parameters	**Site 1**	**Site 2**	**Site 3**
pH	7.72±0.092	7.96±0.078	7.63±0.092
Temperature(^{o}C)	34.85±o.495	38.40±0.849	39.10±0.424
Transparency(cm)	2.10±0.000	2.50±0.000	2.70±0.000
Conductivity(UmhoS/cm)	393.00±2.828	377.00±02.828	356.50±2.121
Dissolved Oxygen(mg/L)	1.90±0.000	2.30±0.000	3.20±0.000
Biological Oxygen Demand(mg/L)	0.90±0.000	0.850±o.0.000	0.90±0.000
Total Dissolved Solids(mg/L)	7.94±0.064	6.84±0.092	11.09±0.156
Sulphate ions(mg/L)	2.10±0.007	1.92±0.042	1.40±0.021
Phosphate ions(mg/L)	0.96±0.035	1.22±0.023	2.11±0.028
Nitrite ions(mg/L)	1.35±0.046	0.90±0.000	1.70±0.000
Nitrate ions(mg/L)	1.05±0.071	1.58±0.021	2.13±0.057
Carbon Content(mg/L)	7.30±0.064	8.14±0.049	8.99±0.120
Oil & Grease(mg/L)	9.10±0.134	9.21±0.035	9.73±0.042
An. gambiae Larval Density(per litre)	17.14/L	14.28/L	12.86/L

Nd= Not Detected

Mean distribution of physicochemical environmental factors across three study zones

Results of the mixed effect linear model showed that the mean distribution of pH, temperature, conductivity DO, BOD, and transparency was not highly significant with p-values 0.163, 0.492, 0.628, 0.234, 0.068 and 0.974 respectively across the three study zones. Likewise, the differences in mean distribution of total dissolved solids, sulphates, phosphates, nitrites, nitrates, carbon content and oil and grease across the three study zones were highly significant (p=0.000). The Bonferoni Post-hoc pairwise comparison tests showed that comparing mean distribution between zone A & B; A & C and B & C for most of the physical environmental factors were not highly significant. For example, for pH, the zone-wise comparisons between zone A against B, A against C and B against C was statistically not significant (p= 0.621, 1.000 and 0.218, respectively). For dissolved oxygen (DO), there were also no statistically significant differences (0.327, 0.620 and 1.000) in mean zone-wise comparisons between zone A against B, A against C and B against C, respectively, while for BOD, A against B, A against C and B against C zone-wise comparisons recorded p-values of 1.000, 0.152 and 0.106 respectively. Lastly, same zone-wise comparisons for temperature, conductivity, and transparency were also not statistically significant (p=1.000). However, the zone-wise comparisons

(A against B, A against C and B against C) for TDS and the environmental chemical factors (sulphates, phosphates, nitrites, nitrates, carbon content and oil and grease) were all statistically significant (p=0.000).

Association between each physicochemical environmental factors and larval density

Preliminary investigation based on bivariate linear regression analysis between larval density and each physical environmental factor show that both pH and temperature were negatively correlated ($p<0.05$) with larval density, while transparency and BOD were positively associated ($p<0.05$) with larval density. The physical environmental parameters that appeared not to influence larval density were conductivity and dissolved oxygen. Furthermore, selected chemical environmental factors (sulphates, phosphates, nitrites, nitrates,) produced a moderate negative correlation ($p<0.05$) with *An. gambiae* larval density while carbon content and oil and grease produced highly significant associations ($p<0.05$) with *An. gambiae* larval density. This suggests that as the levels of these chemical environmental parameters increases, the density of *An. gambiae* larvae decreases. As was observed with dissolved oxygen and conductivity, the total dissolved solids also produced no significant association with larval density.

Combined effect of physicochemical factors on larval density

In order to deduce a model showing a combination of the different physicochemical environmental variables that produce a combined singular effect on larval density, factor analysis followed by regression in principal components was carried out on physicochemical environmental factors and larval density. The SPSS results of the factor analysis showed that the first eight principal components (PC) explained 99% of the variability in the thirteen environmental variables (Fig. 1), and therefore, only these first eight components were retained in the regression analysis. According to the factor loadings, the first component correlated strongly with TDS, sulphates, phosphates, nitrites, and nitrates; PC2 correlated strongly with carbon content and oil and grease, PC3 was explained by conductivity, PC4 by temperature, PC5 correlated with transparency, PC6 was associated with DO, PC7 is explained by pH while PC8 correlated with BOD. Thus, PC1 represents contamination from pesticides and chemical fertilizer application (fertilizer and pesticides contaminants), PC2 represents contamination from the sale, use, processing, and/or discharge of petrochemical/hydrocarbon products (petrochemical contamination) while PC3-8 represent the physical environmental variables.

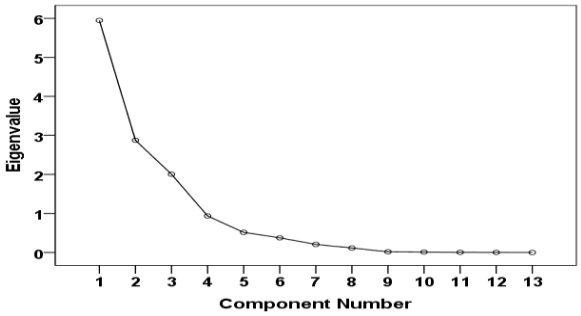


Fig. 1 Scree Plot showing the eigenvalues of the principal components from the factor analysis of the physicochemical environmental variables.

According to this screen plot only the first eight principal components were retained in the analysis.

Finally, the results of the regression in principal components (Table 4) showed that only six out of the eight principal components (PCs) included in the analysis had a significant effect on larval density. The components producing a significant effect on *An. gambiae* larval density in Nigeria comprise pesticides and fertilizer contaminants (PC1), petrochemical/hydrocarbon contaminants (PC2) and the physical environmental variables; conductivity (PC3), temperature (PC4), transparency (PC5), and pH (PC7). In turn, neither DO (PC6) nor BOD (PC8) was found to produce any significant effect on larval density (Table 4).

Table 4 Environmental Physicochemical Factors or Components with Combined Effect on *An. gambiae* Larval Density.

			Hypothesis Test		
Parameter	Coefficient	Std. Error	Wald Chi-square	df	Sig.
Intercept	42.999	0.3749	13156.397	1	<0.001
PC1	-13.2	0.3813	1198.567	1	<0.001
PC2	-22.414	0.3813	3455.792	1	<0.001
PC3	-10.962	0.3813	826.525	1	<0.001
PC4	-5.152	0.3813	182.503	1	<0.001
PC5	12.301	0.3813	1040.873	1	<0.001
PC6	-0.136	0.3813	0.126	1	0.722
PC7	-7.684	0.3813	406.144	1	<0.001
PC8	0.051	0.3813	0.18	1	0.893

Discussion

This study demonstrated that the thriving and density of *An. gambiae* larvae were significantly influenced by some of the physicochemical environmental factors that are associated with mosquito breeding ecologies. While most organisms are known to respond differently to

changes in their physical environments, the choice of the chemical parameters was made based on the most common type of human activities taking place in these zones, but also across rural and urban settings in Nigeria. These activities were considered to be important in contributing to the presence of chemical species in the mosquito breeding habitats. For instance, rain water runoffs from surrounding farmlands in study zone A could bring in high amount of nitrites, nitrates, sulphates and phosphates that are usually present in fertilizers and agro-pesticides, into the mosquito breeding site. Indeed, farmers interviewed nearby these breeding sites confirmed that fertilizers (nitrate and phosphate-base together with organic manure from animal droppings) which both usually contain nitrite, nitrate, and phosphate ions as their major chemical components. Furthermore, 80% of the respondents said they applied various pesticides such as Cypermethrin, Endosulfan, Fipronil, Carbofuran, Dimethoate and Methyl-Parathion. Chemical categorization analysis of these pesticides showed that they belonged to the four major classes of insecticides; carbamates, oganophosphates, organochlorines and pyrethroids and also contain carbon, sulphate and phosphate ions. Furthermore, runoffs from study zone C (petrochemical/hydrocarbon areas) were expected to contain high amount of carbon content and oil and grease. However, since some of these chemical species are also structural constituents of the many chemicals naturally present in the soil, appreciable levels of some of these chemical species were also detected in mosquito breeding sites located in study zone B (residential/domestic environments).

This study revealed that with the exception of oil and grease (which was detected only in zone C), all the breeding sites in the three studied zones contain various levels of these physical and chemical environmental factors. The levels of all the physical factors except BOD were not significantly different across the three study zones. In contrast, the levels of all the chemical environmental parameters varied significantly across the three study zones The levels of TDS, sulphates, phosphates, nitrites and nitrates were significantly higher in sampling sites within study zone A (Table 1). This could be explained by the use of nitrate and phosphates-base fertilizers as well as agro-allied pesticides in farmlands located around these mosquito breeding sites. The levels of carbon content and oil and grease were highest in breeding sites located in study zone C (Table 3), which is consistent with the major types of human activities taking place. There is widespread sale, processing, use and discharge of petroleum products in this zone which appeared to be exacerbated by fuel (petrol or premium motor spirit) vending, a common practice in Northern Nigeria due to constant chronic fuel shortages. In addition, kerosene is a major domestic fuel in Nigeria and is normally sold by small retailers who are usually located within and/or around human habitats. Activities of automobile and motorcycle mechanics, which are often located around human habitation, are also major additional sources of discharged of spent fuel and lubricants into surrounding water bodies and mosquito breeding sites. All these contributed to the presence of very high levels of carbon content and oil and grease in breeding sites located in this zone relative to zones A and B.

The varying degree of *An. gambiae* larval densities from these breeding environments indicates that the physical and chemical environmental conditions of these breeding ecologies produced an effect on larval growth, fitness and survival. The breeding sites located in residential/domestic areas (zone B), which account for the highest transparency and DO and the lowest levels of all the chemical environmental parameters observed, recorded the highest *An. gambiae* larval density. This was consistent with the findings of previous studies, which showed that *An. gambiae* prefers cleaner, clearer and uncontaminated breeding water (Sattler *et al*., 2005). In this study, *An. gambiae* larval density was negatively associated with pH and temperature and positively correlated with transparency and BOD. The majority of the sampling sites recorded temperatures of above 35°C. Thus, this study agrees with previously reported observation that *An. gambiae* larval abundance increases with increasing temperature of up to 28-32°C above which it decreases (Munga *et al*., 2005; Bayoh and Lindsay, 2003). This means that temperatures of above 30°C affect the growth, fitness and survival of *An. gambiae* larvae. This observation is supported by the findings of Atkinson (1994) which showed that growth promoting enzyme-catalysed reactions decrease at high temperatures. The observed effect of pH on *An. gambiae* larval density in this study indirectly agrees with the findings of Ademoroti (2003) which reported that pH increases with increasing temperature due to concentration of carbon dioxide; at higher temperatures the concentration of dissolved carbon dioxide in water bodies increases. This study revealed that *An. gambiae* was more abundant in clearer than in turbid water bodies. This result is similar to the findings of Mwangangi and colleagues (2010) but contradicts the findings of McCrae (1984) which showed that *An. gambiae* females preferred turbid water to clear water for oviposition. The levels of many of these materials are significantly lower in study zone B, as indicated by the concentration of TDS (Table 2) which explains the higher larval density recorded in this study zone. The level of BOD is dependent on the

organic content of a water body. The observed effect of DO and BOD on larval density in this study is consistent with previous observations that higher aquatic organisms thrives better in water bodies with higher BOD than those with lower BOD levels. This is because higher levels of organic compounds lead to increase in microbial population which depletes dissolved oxygen (Goldman and Home, 1983).

The density of *An. gambiae* larvae was negatively associated with the levels of all the chemical factors analysed in this study. This means that increase in the levels of these chemical species lead to decrease in the density of *An. gambiae* larvae. The fact that *An. gambiae* larval density decreases with increasing levels of these chemical species suggest that the mosquito larvae prefers breeding sites low in levels of these chemical species as evidenced by the high larval density recorded in study zone B. However, this may not necessarily equate to developmental tolerance, survivorship and emergence of advanced and fully matured mosquito forms (Osse' *et al*., 2012; Jannat and Roitberg, 2013). The presence of *An. gambiae* larvae in breeding sites containing high levels of chemical species suggest a gradual potential emergence of tolerance of *An. gambiae* larvae to these environmental chemical factors, especially that the relative lower larval densities recorded in zones A (agricultural) and C (petrochemical) in comparison with zone B (domestic/residential) are equally high when compared to other studies (Yadouleton *et al*., 2011; Antonio-Nkondjio *et al*., 2012; Afrane *et al*., 2012). This may constitute a serious threat to the environmental management approaches to controlling malaria vector density. Environmental management strategy that aims to reduce adult vector population by targeting their aquatic immature (egg, larvae and pupae) is emerging as the mainstay of the contemporary approaches to malaria management especially in areas with high vectorial density. This strategy employed the use of various larvicidal chemical compounds to reduce larval density and by extension to minimize adult vector abundance (Gu and Novak, 2005). Therefore, potential tolerance to chemical factors arising from the use of agro-chemicals and petrochemical products reported in this study could pose a significant threat to the effectiveness of various larvicidal agents, especially those with similar structures and activity relationship with the environmental agro and petro-chemical products identified with the breeding sites ecology. In addition, adult mosquitoes emerging from these breeding ecologies could potentially be selected for both intrinsic and acquired resistance to agents used for their control, even in the absence of prior exposure or challenge.

The results of factor and redundancy analysis (Table 4) deduced a model of a combination of physicochemical environmental factors that produced the most significant combined effect on *An. gambiae* larval density. According to this model (Table 4), both pesticide and fertilizer contaminants, petrochemical/hydrocarbon contaminants, together with some of the physical environmental factors produced the most singular combined effect on larval density. This suggests that fertilizer application for soil enrichment could be as important as the use of pesticides for agricultural pest control, in producing potential tolerance to these environmental factors by *An gambiae* larvae. Many previous studies (Vulule *et al*., 1994; Awolola *et al*., 2005) have implicated various agricultural practices, including pesticides application, in the emergence and development of insecticides resistance in malaria vectors. The majority of these studies were restricted to adult mosquitoes and at the level of post-insecticide application. Therefore, there is a need for studies targeting the aquatic life stages at pre-insecticide exposure. Findings from such studies like this present one would complement those of post-insecticides exposure by providing information on the various environmental factors that could serve as potential selection factors for the development and emergence of insecticides resistance.

Conclusion

Findings from this study suggest that *An. gambiae* breeding sites productivity (as measured by larval density) could be under strong influence from the physicochemical environmental conditions of the mosquito breeding sites, and the levels and characteristics of these physicochemical environmental factors are functions of the human related activities taking place within and around the mosquito breeding environments. The impact of these observations on the behaviour of the emerging mosquito, especially their response to the various insecticides-based vector control programmes, could pose serious challenges to malaria management and control. Thus studies on the ecology, development, emergence and evolution of mosquito vectors and the impact of human component of mosquito vector ecology, in more quantitative ways, appeared to be quintessential components for sustainable long term disease management, control, eradication or elimination, which must be supported and promoted.

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