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BIOTIC AND ABIOTIC FACTORS IN *Anopheles gambiae* BREEDING HABITATS AS A POTENTIAL TOOL TO FIGHT MALARIA IN CENTRAL UGANDA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author OH collected the samples, conducted the laboratory work, analysed the data and wrote the first draft of the manuscript. Author OR proof read and guided the writing process. Author CM provided guideline on data analysis and proof read the manuscript. Author KJ conceived the research idea and proof read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: There is a high risk of malaria infection in Uganda due to availability of conducive conditions in breeding habitats of *An. gambiae* s.l., the vectors for *Plasmodium*, the causative agent of malaria.
Aim: The aim of this study was to determine whether the abundance and distribution of macro-invertebrates and *An. gambiae* s.l. are influenced by water physico-chemical parameters.
Methods: In this study, habitats were classified as ponds, streams, temporary pools and roadside ditches. From these habitats, electrical conductivity, total dissolved solids, temperature and pH were measured in-situ in the morning and afternoon between October and December 2017. Macro-invertebrates and *An. gambiae* s.l. larvae were sampled, preserved, morphologically identified and counted.
Results: There was a strong association between *An. gambiae* s.l. with land use, habitat types and water physico-chemical parameters. Baetidae, Coenagrionidae, Aeshnidae, Nepidae, Lymnaeidae and Hirudidae were highly abundant in streams. Notonectidae, Haliplidae and Elmidae were dominant in ponds while Dytiscidae, Culicidae, Chironomidae, Sphaerolichidae and *An. gambiae* s.l. were abundant in temporary pools. Carabidae were abundant in roadside ditches.
Conclusion: Water physico-chemical parameters, land use and habitat types influenced the abundance and distribution of macro-invertebrates including *An. gambiae* s.l. We recommend that studies should be conducted to establish the mechanisms through which these factors influence abundance and distribution of *An. gambiae* s.l. and other macro-invertebrates.

Keywords: *Anopheles gambiae* s.l; abiotic and biotic factors; habitats; River Sezibwa; Uganda.

1. INTRODUCTION

Globally, vector control is an essential component of malaria prevention. Control strategies such as Long Lasting Insecticide treated bed Nets (LLINs) and Indoor Residual Spraying (IRS) have been found to reduce malaria transmission under sufficiently high coverage [1]. However, these approaches target adult

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mosquitoes in residential houses, ignoring the outdoor biting behaviours and the ecology of immature stages of the *An. gambiae* s.l. Control of malaria through exploiting the ecology of immature stages of *An. gambiae* s.l has succeeded in several parts of the world [2,3]. For instance in France, alkaline pH and higher temperature (abiotic factors) and selected macro-invertebrates (biotic factor) reduced the population of *Anopheles arabiensis* [4] which is also a key player in *Plasmodium* (the causative agent of human malaria) transmission in other parts of the world. The abiotic factors such as pH and temperature probably negatively affect the metabolism of mosquitoes. On the other hand, other macro-invertebrates may also compete for food, space while some metabolic products from macro-invertebrates may kill mosquito larvae in a shared habitat.

In sub-Saharan Africa, exploitation of breeding habitats of immature stages of *An. gambiae* s.l. mosquitoes has received insufficient attention and yet, this may generate baseline information that can be used to supplement the currently available approaches in the fight against malaria. The population size of *Plasmodium* vectors is determined by the abundance of immature stages of *An. gambiae* s.l. in different aquatic breeding habitats of a particular community. These breeding habitats have variation in the composition of abiotic and biotic factors. These variations probably influences the association between macro-invertebrates and immature mosquitoes which then, drives their population dynamics.

In order to exploit the abiotic and biotic factors in the immature *An. gambiae* s.l. breeding habitats as a potential tool to reduce population of *An. gambiae* s.l. in the fight against malaria, there is need to understand the ecology of the immature stages of *An. gambiae* s.l. Several studies [5,6,7,4,8,9,10] showed that the biotic and abiotic factors of breeding habitats of immature *An. gambiae* s.l can potentially be exploited as important tools to supress population size of *Anopheles* mosquitoes in the fight against malaria.

In 2016 and 2017, Uganda contributed 4% of the total 445 thousand deaths due to malaria in sub-Saharan Africa [13]. This has negatively affected the socio-economic activities of the local population. Paucity of information exist in regards to the possible abiotic and biotic factors in the *An. gambiae* s.l breeding habitats that may potentially regulate population of the *Plasmodium* vectors. Efforts put towards bridging this gap, may contribute to the good health and well-being of the target population as stated under the third United Nation sustainable development goal. Here, we investigated the influence of selected water physico-chemical parameters on association between the abundance of macro-invertebrates and *An. gambiae* s.l larval densities across habitat types in Kibuye and Kayonjo villages located along River Sezibwa, Uganda.

2. MATERIALS AND METHODS

2.1 Study Area

This study was a longitudinal survey conducted between October and December, 2017 in Kibuye and Kayonjo villages located along River Sezibwa, Uganda. Kibuye is located on the western side of the river bank while Kayonjo is on the eastern in Mukono and Kayunga district respectively (Fig. 1). Kibbuye and Kayonjo are approximately 33 kms apart and approximately 68 and 81 kms from Kampala respectively. Mukono lies within coordinates of 0°21'17.99" N, 32°45'07.57" E in Uganda with a total human population of 596,804 on 1,875.1 km^2 of land cover [11]. Kayunga lies within coordinates of 0°59'09.67"N, 32°51'12.87" E with a total population of 294,613 on 1,810 km^2 of land cover. Both districts are predominantly covered by savannah vegetation [12] and experience bimodal rainfall patterns with average annual temperature of 21.5°C [13]. The main economic activities in the study area are fishing and subsistence agriculture.

2.2 Mosquito Habitat Characterization

Preliminary field surveys were conducted in Kibuye and Kayonjo villages in July, 2017 to identify potential breeding habitats of *An. gambiae* s.l. mosquito larvae and macro-invertebrates. Based on the survey results, four habitat types of less than three meters in depth where identified in each village and categorised as: streams (i.e., naturally slow running water bodies with diversions of stagnant water from the main stream which may not dry off in absence of rainfall); ponds (non- flowing water collected in man-made pools that may not dry off within four months in the absence of rainfall); temporary pools (non-flowing water collected in small pools that may not dry off in two months without rainfall) and roadside ditches (non-flowing water collected along the pavement that may persist for at least two months without rainfall) (Fig. 2). Geographical coordinates of habitats were recorded using a Global Positioning System (Shenzhen Pengjin Technology Co., Ltd, Shenzhen China) receiver.

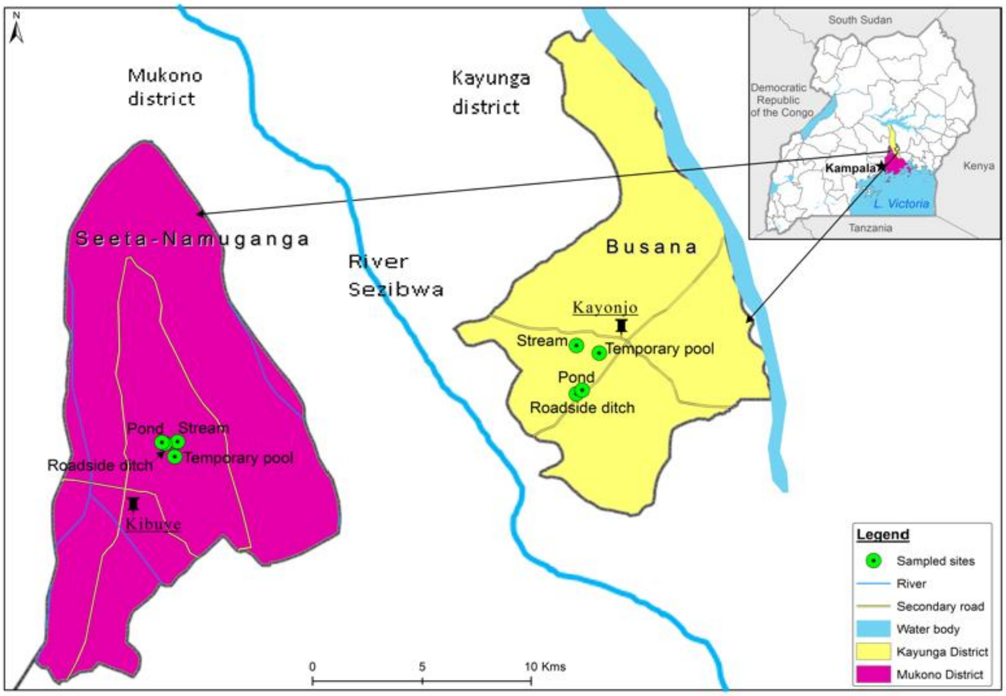

Fig. 1. Location of sampling points in Kibuye and Kayonjo villages

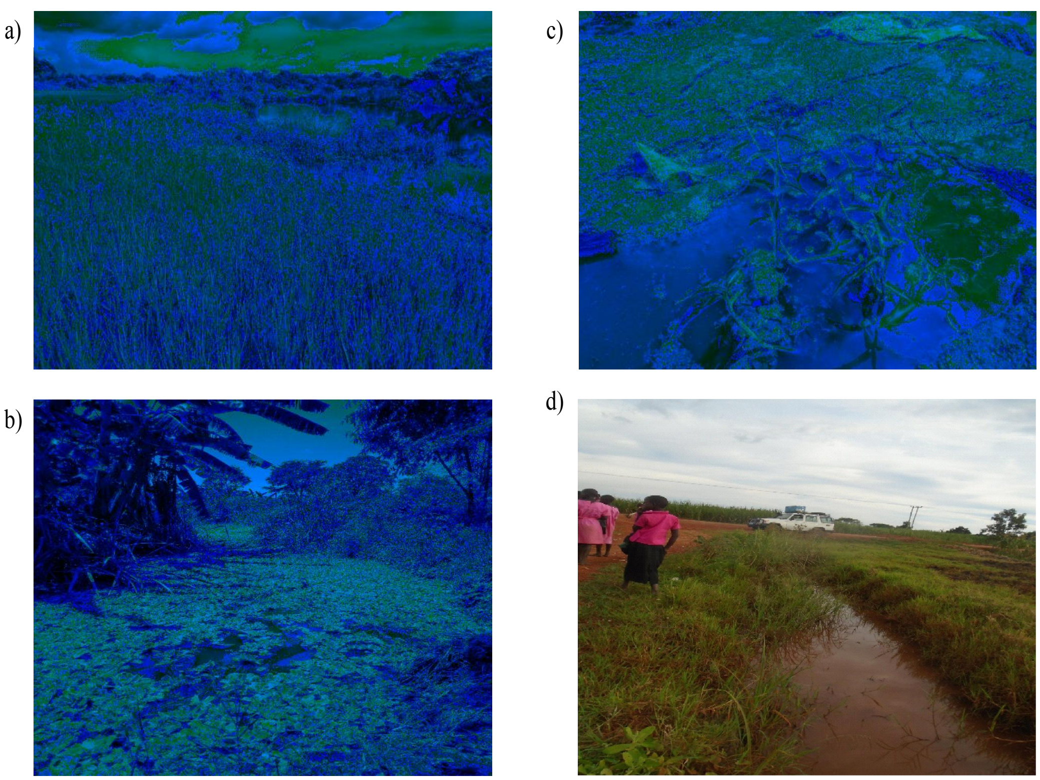

Fig. 2(a-d). a) Stream; b) Roadside ditch; c) Pond and d) Temporary pool mosquito and macro-invertebrate breeding habitats

2.3 Determination of Water Physico-chemical Parameters

Electrical conductivity (EC) (μS/cm), total dissolved solids (TDS) (mg/L) and temperature (°C) were determined *in-situ* using a pH/EC/TDS Combo testing meter (Hanna Instruments, Woonsocket, RI, U.S.A.) after calibration. These were conducted in the randomly determined sampling sites in each geo-referenced similar habitat. The measurements were done in the morning (1000 -1100 hrs.) and afternoon (100-200 hrs.) by dipping the probe into the water followed by recording readings. The same procedures were followed for determination of pH using a hand held meter.

2.4 Sample Collection, Identification and Quantification

From each breeding site where physico-chemical parameters were determined, dominant land use types were recorded and four dips using standard 350 mL dippers (Bio Quip Products, Inc. California, USA) were used to sample macro-invertebrates and *An. gambiae* s.l. mosquito larvae. The mosquito larvae were siphoned using standard 50 mL aquatic pippetes attached to rubber bulbs and transferred into 15 mL falcon tubes containing 4 mL of 80% ethanol. The remaining contents were sieved using stainless steel mesh strainer, macro-invertebrates sorted and placed in different 5 mL falcon tubes containing 8 mL of 80% ethanol. Samples were transported to the Entomology laboratory at Uganda Virus Research Institute (UVRI), Entebbe and morphologically identified to family level using a light microscope (X 40), identified following a guide [14] and enumerated.

2.5 Data Analysis

Data obtained were analysed using predictive analysis software (PASW)/ SPSS version 21.0 for windows (SPSS Inc., Chicago, IL). Shapiro-Wilk test for normality was performed and consequently a non-parametric spearman's rank correlations test was conducted to determine the association of *An. gambiae* s.l. larval density with abundance of different macro-invertebrate taxa. Kruskal-Wallis test was performed to compare water physico-chemical parameters across habitat types. The same test was conducted to compare the association of *An. gambiae* s.l. larval density with abundance of macro-invertebrates across habitat types.

Regression models were built modelling the presence and abundance of *An. gambiae* s.l. in different habitat types. The goal was to build models indicating whether *An. gambiae* s.l. larvae were present or not, and another model indicating the number/abundance of *An. gambiae* s.l. larvae when they were present. From the original dataset, two datasets were created. One indicating whether *An. gambiae* s.l. larvae were present or not, and the other showing the abundance when they were present. Dummies were created for the categorical variables. Forward stepwise logistic regression was used to model the presence of *An. gambiae* s.l. larvae. To model the abundance of *An. gambiae* s.l. when they were present, forward stepwise linear regression was conducted. To fulfil all underlying assumptions of the models, log transformations of the outcome variables were done in each case. A multivariate analysis using Principle Component Analysis (PCA) was used to establish the relationship between pH, TDS, EC and temperature with macro-invertebrates and *An. gambiae* s.l.

3. RESULTS

3.1 Association between *An. gambiae* s.l. Larval Densities and Abundance of Macro-invertebrates across Habitat Types

Association of *An. gambiae* s.l. larval density with abundance of macro-invertebrates varied significantly across habitat types investigated ($\chi 2$: = 325.7, df = 3, n =767, P < .001). For instance in ponds, significant positive correlations between *An. gambiae* s.l. larval density with abundance of water scorpion (Nepidae) (rho = 0.54, n = 192, *P* < .001), Snails (Lymnaeidae) (rho = 0.30, n = 192, *P* < .001), culex (Culicidae) (rho = 0.51, n = 192, *P* < .001), damselflies (Coenagrionidae) (rho = 0.70, n = 192, *P* < .001), diving bell spider/ water spider (Cybaeidae) (rho = 0.71, n = 192, *P* < .001), riffle beetles (Elmidae) (rho = 0.39, n = 192, *P* = < .001), predacious diving beetles (Dytiscidae) (rho = 0.66, n = 192, *P* < .001), (backswimmers) Notonectidae (rho = 0.21, n = 192, *P* = .02) and whirligig beetles (Gyrinidae) (rho = 0.16, n = 192, *P* = .03) were recorded. On the other hand, significant negative correlations with abundance of mayflies (Baetidae) (rho = - 0.71, n = 192, *P* < .001), dragonflies (Aeshnidae) (rho = - 0.30, n = 192, *P* < .001), water striders (Gerridae) (rho = - 0.64, n = 192, *P* < .001), midges (Chironomidae) (rho = - 0.18, n = 192, *P* < .001) and crawling water beetles (Haliplidae) (rho = - 0.38, n = 192, *P* < .001) were also recorded in the same habitat.

In streams, significant positive correlations were recorded between *An. gambiae* s.l. larval density with abundance of Chironomidae (rho = 0.24, n = 192, *P* < .001) while significant negative correlations were recorded with abundance of Coenagrionidae (rho = - 0.17, n = 192, *P* = .02) and Notonectidae (rho = - 0.14, n = 192, *P* = .05). In temporary pools, significant positive correlations were recorded between *An. gambiae* s.l. larval density with abundance of Aeshnidae (rho = 0.17, n = 192, *P* = .02) and Dytiscidae (rho = 0.15, n = 192, *P* = .04) while significant negative correlation was recorded with abundance of Baetidae (rho = - 0.23, n = 192, *P* = .02).

In roadside ditches, significant positive correlations were recorded between *An. gambiae* s.l. larval density with abundance of Lymnaeidae (rho = 0.15, n = 192, *P* = .05), Chironomidae (rho = 0.67, n = 192, *P* < .001) and Dytiscidae (rho = 0.45, n = 192, *P* < .001) while the abundance of Baetidae (rho = - 0.15, n = 192, *P* = .04) and ground beetles Carabidae (rho = - 0.22, n = 192, *P* = .02) were significantly negatively correlated with *An. gambiae* s.l. larval densities.

Multivariate analysis by PCA extracted two principal components that explained 81% of the observed variance in the association of macro-invertebrates and *An. gambiae* s.l. larvae. Component 1 explained 52% while component 2 explained 29% .Temperature, pH, total dissolved solids (TDS) and electrical conductivity (EC) of water explained variations in component 1. However, variations in component 2 could not be explained by variations in electrical conductivity.

Hirudidae, Nepidae, Gerridae, Aeshnidae, Coenagrionidae and Baetidae loaded strongly on component 1, whereas Elmidae, Notonectidae, Gyrinidae, Haliplidae, Cybaeidae, Hydrophilidae, Dytiscidae loaded strongly on component 2 (Fig. 3). Nepidae, Baetidae, Coenagrionidae, Aeshnidae, Hirudidae, Cybaeidae, Haliplidae, Elmidae, Notonectidae, Hydrophilidae and Carabidae negatively associated with temperature, pH, TDS and EC. On the other hand, Culicidae, Chironomidae, Sphaerolichidae, Dytiscidae, Gyrinidae, Gerridae, and *An. gambiae* s.l. larvae were positively associated (Fig. 3).

3.2 Relationship between *An. gambiae* s.l., Habitat Types, Macro-invertebrates and Water Physico-chemical Parameters

A binary logistic regression model developed to predict the presence of *An. gambiae* s.l. gave a Nagelkerke R^2 value of 0.368 implying that the variables included in the model was able to explain 36.8% variance in the model. The findings indicate

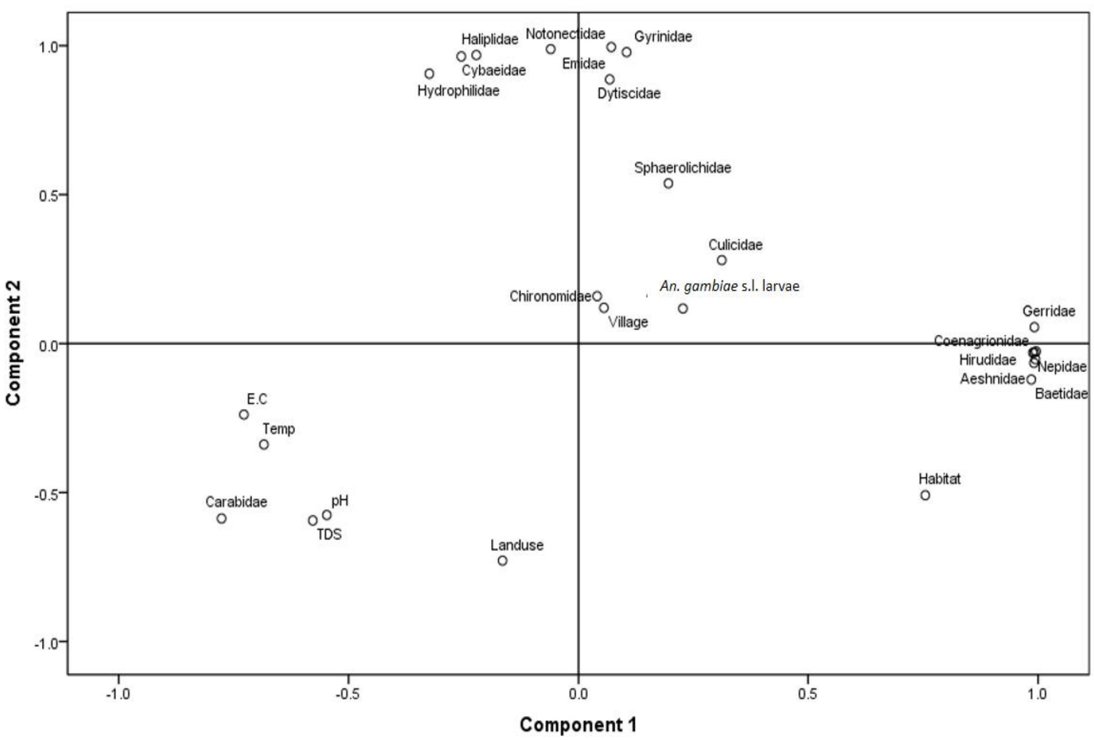

Fig. 3. Relationship between water physico-chemical parameters with macro-invertebrates and *An. gambiae* s.l. larvae

that the variables included in the model predicted the presence of *An. gambiae* s.l. in the study sites (χ^2 = 225.54, (df = 6), P < .001). Change of habitats from roadside ditches to temporary pools increased the chances of getting *An. gambiae* s.l. by 0.37 times (Table 1).

The linear regression model developed explained 62.2% variance in *An. gambiae* s.l. abundance. Factors included in the model were: habitats, land use, temperature, pH, EC, TDS, Nepidae, Baetidae, Coenagrionidae, Aeshnidae, Hirudidae, Gerridae, Lymnaeidae, Culicidae, Chironomidae, Sphaerolichidae, Cybaeidae, Haliplidae, Elmidae, Dytiscidae, Notonectidae, Carabidae, Gyrinidae, and Hydrophilidae. All together, these factors significantly explained *An. gambiae* s.l. abundance (F (84.51) = 5.61, P < .001). However, TDS, habitat types, land use, temperature, pH, Baetidae, Aeshnidae, Hirudidae, Lymnaeidae, Culicidae, Chironomidae, Haliplidae, Cybaeidae and Dytiscidae only significantly predicted *An. gambiae* s.l. abundance (Table 2). These results were also supported by PCA (Fig. 3).

There were significant differences in water physico-chemical parameter across habitats: Temperature; (χ^2: =710.7, df = 3, n= 4, *P* < .001), pH; (χ2:= 638.2 , df = 3, n =4, *P* < .001), TDS; (χ2: =550.4, df = 3, n = 4, *P* < .001) and EC (χ2: =553.9, df = 3, n =4, *P* < .001). Differences were recorded among all the determined water physico-chemical parameters of habitats.

Table 1. Logistic regression model predicting presence of *An. gambiae* s.l. in the study sites

Factors	B	S.E.	Exp(B)	P
Land use	-5.64	1.30	0.04	< .001
Roadside ditches	-5.37	1.77	0.05	.02
Temporary pools	-0.99	0.49	0.37	.04
Temperature	0.47	0.19	1.61	<.001
pH	0.56	0.16	1.75	< .001
EC	-0.06	0.01	0.94	< .001
TDS	-0.01	0.04	0.10	.13

χ^2 = 225.54, df = 6, P = < .001; Nagelkerke R^2 = 0.368

Table 2. Linear regression model predicting abundance of *An. gambiae* s.l. in the study sites

Factors	Beta	T	P
Habitats	0.55	11.33	< .001
Land use	-0.13	-2.31	.02
Temperature	0.18	3.30	.01
pH	0.23	2.82	.05
EC	-0.04	-0.93	.35
TDS	-0.15	-3.62	< .001
Nepidae	-0.01	-0.12	.90
Baetidae	-0.66	-8.10	< .001
Coenagrionidae	-0.02	-0.03	.97
Aeshnidae	0.28	5.92	< .001
Hirudidae	0.17	4.93	< .001
Gerridae	-0.14	-2.67	.08
Lymnaeidae	0.28	5.78	< .001
Culicidae	0.07	2.36	.02
Chironomidae	0.14	5.13	< .001
Sphaerolichidae	0.02	0.52	.61
Cybaeidae	0.15	3.72	< .001
Haliplidae	-0.11	-2.58	.01
Elmidae	-0.06	-1.44	.15
Dytiscidae	0.21	3.82	< .001
Notonectidae	0.03	0.05	.96
Carabidae	0.01	0.27	.78
Gyrinidae	0.04	1.01	.31
Hydrophilidae	-0.05	-1.64	.10

F (84.51) = 5.61, P < .001. Italicized variables are statistically significant

Table 3. Mean and standard deviation of water physico-chemical parameters across habitats

Habitat	Tempt (°C)	EC (uS/cm)	TDS (mg/L)	pH	Macro-invertebrate families
Streams	26.3 ± .53	366.4 ± 36.75	470.9 ± 3.66	3.8 ± .51	Baetidae, Coenagrionidae, Aeshnidae, Nepidae, Lymnaeidae and Hirudidae
Ponds	27.9 ± .44	402.2 ± 10.31	478.4 ± 3.39	4.3 ± .39	Notonectidae, Haliplidae and Elmidae
Temporary pools	29.0 ± .28	415.3 ± 12.52	522.8 ± 2.06	6.4 ± .96	Dytiscidae, Culicidae, Chironomidae, Sphaerolichidae and *An. gambiae* s.l.
Roadside ditches	30.8 ± .75	441.3 ± 29.47	556.6 ± 4.10	8.1 ± .48	Carabidae

Macro-invertebrate families included in the table were significantly abundant in habitats with the corresponding values of determined water physico-chemical parameters

The highest mean temperature were recorded in roadside ditches followed by temporary pools, ponds and least in streams. The same trend were recorded with pH, EC and TDS. Under low temperature such as in streams and ponds, pH was slightly acidic but alkaline under high temperature in temporary pools and roadside ditches. Further, EC and TDS values were low in streams and ponds but high in temporary pools and roadside ditches (Table 3).

4. DISCUSSION

Kibuye and Kayonjo villages continuously experience prevalence of malaria and genetic diversity of *An. gambiae* s.l. among the districts of central region in Uganda. This provides focus for investigations on the biotic and abiotic composition of the breeding habitats of *An. gambiae* s.l. which is the principal vector of *Plasmodium* responsible for the cause of malaria in this region (Target Malaria Project, UVRI un-published data). Results presented herein provide the first attempt at national level to understand how abiotic and biotic factors interact to influence the distribution of *An. gambiae* s.l. larvae across habitat types.

Aquatic insects are sensitive to physico-chemical changes of water in their breeding habitats, since physiological processes in aquatic insects operate within defined ranges [15]. Therefore, ecological factors such as water physico-chemical parameters and macro-invertebrates compositions of *An. gambiae* s.l. breeding habitats is worth investigating in search for potential alternative control strategies for malaria causing vectors.

Human activities in different habitat types influences water physico-chemical parameters which may determine the presence or absence of *An. gambiae* s.l. in a given area. In this study, temporary pools had higher distribution of *An. gambiae* s.l. This is probably due to their exposure to sunshine and rapid increase of temperature owing to the small water volume which enable quick convectional transfer of heat within water molecules [16]. This study has further shown that *An. gambiae* s.l., Culicidae, Chironomidae prefer to breed in water with temperature ranging from 29-30°C and pH of 6-7 [17].

Linear regression and PCA analysis showed that TDS, habitat types, land use, temperature, pH, and some macro-invertebrates (Baetidae, Aeshnidae, Hirudidae, Lymnaeidae, Culicidae, Chironomidae, Haliplidae, Cybaeidae and Dytiscidae) were associated with *An. gambiae* s.l. Different aquatic habitats have varying physico-chemical conditions which may influence how different macro-invertebrates including *An. gambiae* s.l. larvae interact in shared habitats. For instance, pH, temperature and TDS affect enzymatic activities of different insects which may alter their growth and consequently determining their abundance and distribution [15]. The abundance of Baetidae, Aeshnidae and Hirudidae were negatively correlated with pH, TDS and temperature. These group of macro-invertebrates (Baetidae, Aeshnidae and Hirudidae) were mainly sampled from streams and ponds where pH, TDS and temperature values were low while Culicidae, *An. gambiae* s.l., Chironomidae, Haliplidae, Cybaeidae and Dytiscidae that were positively associated with pH, TDS and temperature mainly were from temporary pools. Most temporary pools had shallow depth and the dominant soil type was clay. Further, domestic animals were being grazed around mosquito breeding habitats. Clay particles dislodged by animal hooves is responsible for the high TDS which increase the electric conductivity of water [18,19].

In streams and ponds, water temperature, TDS and pH were low. Such conditions favoured Baetidae, Coenagrionidae, Aeshnidae, Nepidae, Lymnaeidae, Hirudidae, Notonectidae, Haliplidae and Elmidae. Physiological process of Baetidae, Coenagrionidae, Aeshnidae, Nepidae, Lymnaeidae, Hirudidae, Notonectidae, Haliplidae and Elmidae performs better under low water temperature, TDS and pH [15,20]. On the other hand, Dytiscidae, Culicidae, Chironomidae, Sphaerolichidae, *An. gambiae* s.l. and Carabidae were highly abundant in temporary pools and roadside ditches which had high pH, TDS and temperature implying that their abundance and distribution is favoured by these conditions [21,17]. Ponds and streams were surrounded by Eucalyptus trees, rice and cabbage gardens. Coupled with probable use of insecticides to spray crops, Eucalyptus leaves lowers the pH of water [22] which could have contributed to the acidic pH of the streams and ponds. It's possible that acidic water with low temperature, TDS and EC negatively affect survival of *An. gambiae* s.l., Culicidae, Chironomidae, Haliplidae, Cybaeidae and Dytiscidae.

5. CONCLUSION

Habitat types, high temperature, pH and some group of macro-invertebrates (Aeshnidae, Hirudidae, Lymnaeidae, Culicidae, Chironomidae, Cybaeidae and Dytiscidae) predicted the abundance of *An. gambiae* s.l. larvae in breeding habitats of the study sites. *An. gambiae* s.l. were mainly distributed in temporary pools compared to other habitat types. On the other hand, land use types, TDS, Baetidae and Haliplidae were negatively associated with the abundance of *An. gambiae* s.l. Baetidae, Coenagrionidae, Aeshnidae, Nepidae, Lymnaeidae and Hirudidae were distributed and abundant under water physico-chemical conditions of streams while Notonectidae, Haliplidae and Elmidae were abundant under conditions in ponds; Dytiscidae, Culicidae, Chironomidae, and Sphaerolichidae. *An. gambiae* s.l. were mainly distributed in temporary pools. Alkaline pH and high temperature of the temporary pool were favourable to *An. gambiae* s.l. larvae hence its high abundance. The findings reported herein provide new information on how water physico-chemical parameters influences the abundance and distribution of *An. gambiae* s.l. larvae and macro-invertebrates in breeding habitats in selected villages along River Sezibwa which can potentially be useful to any control approach targeting larval stage of *An. gambiae* s.l. in the fight against malaria.

Since this study was conducted only in one season (wet), we recommend further studies to consider sampling of macro-invertebrates and *An. gambiae* s.l. in both wet and dry seasons to establish seasonal influence of water physico-chemical parameters on abundance and distribution of *An. gambiae* s.l. and macro-invertebrates across habitat types and also to determine temperature and pH ranges that can be lethal to *An. gambiae* s.l. larvae in temporary pools and roadside ditches.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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