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Assessment of Anopheles Larval Source Reduction Using Cow Dung: Environmental Perspective on Pro-poor Tool for Malaria Vector Control

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ABSTRACT: This paper presents the results of a field experiment, whose aim was to investigate the potential of dissolved cow dung to cause anopheles larval population reduction in Yala swamp, western Kenya. Field experiments were conducted in two fish ponds located within the drained part of a wetland, and two swamp pools in undisturbed parts of the wetland. The experimental pond was treated with decomposed cow dung, while control pond and swamp pools were not treated. Data collection involved regular larvae sampling and water quality measurements in order to compare mosquito larval densities among the habitats based on variations in physic-chemical parameters. The species of anopheles mosquitoes identified were *Anopheles gambiae* complex and *Anopheles funestus.* The distribution and abundance of mosquito larvae was significantly associated with water quality parameters such as pH ($r = -0.48$; $P < 0.01$), DO ($P < 0.01$), Conductivity ($r = -0.11$; $P < 0.01$) and Turbidity ($r = -0.57$; $P < 0.01$). The results show that cow-dung treatment significantly reduced *Anopheles* species population in the experimental pond without diminishing the dissolve oxygen concentration levels required for diverse biota. Thus, cow-dung has potential to control aquatic stage of malaria vectors, and further experiments could help to refine its use as a tool for larval source reduction in rural settings. This can facilitate community-based vectors control in rural areas where numerous transient mosquito breeding habitats occur.

KEYWORDS: Anopheles larvae, Source reduction, Cow-dung treatment, Water quality, Yala Swamp.

1 BACKGROUND

Malaria is a hurdle in achieving millennium development goals, as it impedes global effort aimed at promoting overall human development in the developing countries, especially sub-Saharan Africa. The disease is a major public health problem to the global community. Evidently, the burden of malaria on the human society is felt disproportionately between developed and developing nations, urban and rural populations [1], [2], as well as within household disparities between men and women, children and adults [3], [4]. In Africa, malaria remains predominant in the rural areas of sub-Saharan region [5], [6], where the poor have very limited financial assets and consequently fare worse when malaria attacks and persists [7]. It is estimated that malaria deprives Africa resources worth U.S $ 12 billion every year [8]. This means that Africa's over reliance on conventional approaches to malaria control, which hugely targets the parasites and adult mosquito vectors, is not yielding much needed results with visible socio-economic land marks. Research focus on a set of interventions for malaria that integrate larval source reduction rather than pursuing the conventional approach is now required.

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Larval source reduction is a very old mosquito vectors control strategy, and has worked so well in efforts to eradicate malaria in developed countries [9]. This approach, however, has not been successful in Africa [10]. For decades in the United States of America (US), Canada, throughout Europe, Brazil and Singapore, larval control yielded positive results for malaria control [11], [12]. Strategies such as marsh drainage, ditch clearing, use of larvivorous fish, (Gambusia affinis), intermittent irrigation and *Bacillus thuringiensis var. israelensis* (Bti) yielded positive results in Italy, USA, Israel, Indonesia and some parts of Brazil [9], [10], [13]. Most of these approaches are expensive and hence require huge sums of money to undertake on a large scale. A strong view is emerging that larval source reduction strategies used in developed countries may have succeeded due to the level of industrialization in those countries, and consequent urbanization. This is likely so, because studies have shown that the popular approaches that have been used to reduce suitable mosquito breeding places in urban areas are practically inappropriate in rural settings [14], [15], [16]. However, it is a common knowledge that the highest percentage of African population resides in rural areas. Thus, any mosquito control initiatives that can bear significant reduction of malaria transmission, in Africa, should consider pro-active involvement of the rural population as crucial entry point.

As happens in most African rural settings, mosquitoes' breeding sites are often small, numerous, scattered and shifting with the season and indiscriminate human activities [17]. Elimination of mosquito breeding in majority of such wide variety of temporary water collections, over sufficiently large area, will require active involvement of the entire community. Ultimately, this cannot be achieved without fronting a supplementary tool for larval control that is simple, cheap, effective, less labor intensive, environment friendly and culturally acceptable.

Further, recent studies show that malaria is declining in some African countries [18], and this is attributed to integrated malaria control approach employed for the last few decades. The approach involved advocacy for and use of insecticide treated mosquito nets (ITNs), use of indoor residual spray (IRS) as well as timely diagnosis and effective treatment. In this regard, renowned scholars now call for new intervention initiatives that could be added to the existing vector control strategies in order to facilitate speedy eradication of malaria on the continent [19]. Furthermore, the above global malaria control strategies are targeted at the parasites (pathogen) and the adult mosquito vectors which maintain the ecological cycle of malaria transmission [20], [21]. Recently some scholars have expressed their great concern that larval source management has been long neglected in Africa, despite being one of the oldest malaria control strategies [10]. Accordingly, it is widely acknowledged that less effort has been turned on mosquito larval control in developing countries and Africa in particular. A recent study recognized that in order to maintain the gains made in the fight against malaria, while pursuing further reduction of malaria transmission, there needs to be additional tools for vector control [22].

The existing mosquito larval control measures require trained personnel and are generally expensive. Thus, relying entirely on the current approaches to larval source management might continue to alienate communities from participating in larval control programmes. Evidence shows that *Bacillus thuringiensis var. israelensis* (Bti) is expensive, requires frequent repeat applications and cannot control pupae [23], [24]. Even the use of fish predator for mosquito larvae control might only work in relatively large and less transient water pools, which are usually less preferred for anopheles breeding. What is more, serious concerns have been raised about the threats of exposure to chemicals when people use indoor residual sprays such as DDT. What is more, studies have also demonstrated that DDT use resulted in the emergence of resistance strains of mosquitoes, with potential negative effects on biodiversity [25].

Overall, despite the challenges, a renewed call for large scale larval source reduction is lately gaining momentum [26], [27], [28]. This is contrary to pessimists' opinion that an area specific approach might bear results [29], [30]. Both approaches have their own strengths and weaknesses, but most importantly implementing either approach still presents an uphill task especially in Africa. This is so, because distribution of larval habitats and their potential for anopheles larvae productivity varies widely between regions at different spatial and temporal scales [31], [32]. An area wide treatment with larvicides requires greater care not to harm the wider aquatic ecosystem compared to an area specific treatment. On the contrary any area specific treatment necessitates accurate information on productive anopheles larval habitats, which at the moment is not refined [33]. It is on this background that this paper presents the preliminary findings of an innovative research evaluating the potential of decomposed cow-dung to hinder anopheles larval production as a strategy to reducing malaria transmission.

Therefore, the use of cow-dung is aimed at fostering area wide control of mosquito breeding grounds, using locally available material without any financial constraints or technicalities, thereby allowing active involvement of communities in the fight against malaria. This paper presents the evaluation of physic-chemical parameters of dissolved decomposed cow-dung treated pond and their effect on Anopheles mosquito breeding in Yala swamp wetland of western Kenya.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The study was carried out in Yala swamp, Siaya district of Western Kenya. The district lies between latitude 0° 26 South to 0° 18 North and longitude 38° 58′ east and 34° 34′ west. Yala swamp is the third largest deltaic wetland in Kenya after the Lorian swamp and Tana River delta. The swamp has been massively encroached for agricultural purposes. Commercial agriculture goes on in the wetland despite the fact that it is the most valuable riparian and floodplain wetland in the delta of River Yala within the Lake Victoria ecosystem, covering a geographical area of 17,500 ha (175 km²). This raises fear that changing land use system may create suitable habitats for Anopheles mosquito breeding, thereby increasing malaria prevalence in a region that is remote, poor and has ill-equipped health facilities.

2.2 EXPERIMENTAL DESIGN

Study sites were selected for regular sampling of mosquito larvae from two ponds and two small swamp pools in an experimental design. Study sites were identified and demarcated in March 2006 followed by establishment of experimental and control ponds in drained parts of the wetland. The small swamp pools were identified in undisturbed parts of the wetland for regular larvae and water sampling. Experimental and control ponds, each measuring 300 cm x 400 cm at a depth 75 cm, were constructed. Each pond was then filled with fresh water to capacity and left for free flying mosquitoes to colonize and breed. The yala River water was pumped into the ponds from farm irrigation channel using generator. The experimental pond was treated with dissolved decomposed cow dung before the commencement of mosquito larvae sampling in April. Approximately 2kg of decomposed cow-dug was added to 10 litres of water in a plastic tub and mixed thoroughly before extracting the filtrate for the pond treatment (Table 1).

Table 1. Pond treatment with dissolved decomposed cow dung against mosquito larvae

Species targeted	Material used	Application rate-unit/9m³ of pond water	Duration (Days to re-treatment)
An. gambiae *An. funestus*	Dissolved decomposed cow dung	10 Litres	15 days

Source: Field experiment adopted modified version [34].

The pond treatment with cow dung (organic fertilizer) was informed by the study findings of Garg and Bhatnagar [35], which investigated the effect of five different doses (5 000, 10 000, 15 000, 20 000, 24 000 kgha^{-1} year^{-1}) of cow dung on pond productivity and fish biomass in still water ponds. A third dose of 15000 kgha^{-1} year^{-1} yielded higher species diversity (zooplankton and phytoplankton) and fish biomass. It was also observed that dissolved oxygen remained significantly high at 4.7 mgl^{-1}, but the decline of the above parameters occurred with the increase of treatment doses (20000-24000 kgha^{-1} year^{-1}). It was further observed that, at the application rate of 15000 kgha^{-1} year^{-1}, the residual effect of cow dung decreased after 60-75 days. The duration of experiments we carried out between seasons 9wet and dry) fell within the number of days required to minimize residual effects of cow dung. Going by the rate of cow dung application Garg and Bhatnagar used [35], a pond measuring 12 m² requires 18 kg.

2.3 DATA COLLECTION

2.3.1 MOSQUITO LARVAE SAMPLING

Regular mosquito larval sampling was carried out in three categories of habitats (Control pond, treatment pond and swamp pools which occurred in undisturbed parts of the wetland) within the study area. Mosquito larvae were sampled twice a week from April 2006 to August2006 between 8.00 hours and 12.00 hours. Larvae in third and forth instar stages of mosquitoes' development were collected by dipping (using standard dipper). The dipper of capacity 400 ml was used as a survey tool for sampling mosquito larvae as recommended by Claudia [36] and Service [37]. Dipping was done at the rate of 6 dips per m² of water surface area. This was applied in small swamp pools where two such pools were identified and marked for regular sampling. For large habitats (control pond and treatment pond) 30 dips were taken within 5 m² areas along the edges and around floating substratum on water surface. During larvae sampling, vegetation and any substrate on water surface of each habitat was noted on every sampling day for the whole period of study. They included dense mats of floating vegetation, *Cyperus papyrus*, *Typha*, grasses and herbaceous plants. We also noted potential predators of mosquito larvae during the study. These included water bugs (*Diplonychus* sp, Hemiptera: Notonectidae), water beetles and water beetle larvae (*Dytiscus* sp, Coleoptera: Dytiscidae), dragonfly larvae (*Pantala* sp, Odonata: Libellulidae), as well as Toads and tadpoles (Table 2).

Table 2. Mean bio-physical conditions of mosquito larval habitats during the study period

Habitats	Bio-physical Conditions of Larval Habitats		Water Conditions	
	Vegetation	Predators	Colourless & foul	Coloured & foul
Control pond	40(3.79±0.12)	40(2.75±0.11)	†	
Treatment pond	40(4.01±0.22)	40(3.15±0.26)		††
Swamp pools	40 (4.65±0.28)	40(3.08±0.29)	††	

Values on predators and vegetation are given in mean frequency and standard deviation of occurrence in each habitat, while water condition is presented using symbol (**†** for a single condition: colour without smell, **† †** for both conditions: colour and smell).

2.3.2 PHYSICO-CHEMICAL VARIABLES

Selected habitats namely control pond, treatment pond and swamp pools were positive for mosquito larvae, and were consequently evaluated for physico-chemical characteristics. A water sample was collected from each breeding site concurrently with the collection of mosquito immature, between 08:00hrs and 12:00 hrs on each sampling day. Key water quality parameters measured were phosphorus ($PO_4^{-}P$), nitrogen ammonia (NH_4 - N), nitrate ($NO_3^{-}N$), temperature (ºC), dissolved oxygen (DO), hydrogen ion concentration (pH), electrical conductivity (EC) and turbidity. Initial readings were made from a freshly collected water sample in the dipper itself after sorting the larvae and were analyzed within 3-5 hrs of collection in Lake Victoria Environment Management Programme (LVEMP) laboratory, Kisumu. Elsewhere in Pakistan, a study demonstrated no significant differences in physico-chemical parameters of mosquito breeding water within 12 hours after collection [38]. Thus, it was assumed that any significant physico-chemical changes would not occur within the short time lag between collection and analysis in the LVEMP Laboratory. However, due to long distance and lack of reliable public transport from the study area to Kisumu town where LVEMP Laboratory is located, only parameters that could be measured directly in-situ were tested further during larval sampling. Accordingly, three of the initial eight parameters measured ($PO_4^{-}P$, NH_4 - N, and $NO_3^{-}N$) were not tested further (Table 3).

The Temperature, pH, Conductivity, Dissolved Oxygen (DO) were measured using M90 multi-probe meter. Turbidity was measured in Jackson Turbidity Units (JTU), Renn CE [39]. Temperature (ºC) and hydrogen ion concentration (pH) were determined in-site at the time of collection, die latter using BDH pH-paper. Other parameters such as dissolved oxygen, turbidity and conductivity were analyzed during field sampling exercise using M90 multi-probe meter. Turbidity was measured in Jackson turbidity units (JTU).

Table 3. Data of water quality parameters tested in Lake Victoria Environment Management Programme Laboratory, Kisumu.

Parameters	Date Sampled	**Larval Habitats** Control Pond	Treated Pond	Swamp pool	Unit of Measurement
Temp.	26.04.2006	26	26.3	26.7	ºC
pH	26.04.2006	6.7	5.8	7.5	pH scale
COND	26.04.2006	108.1	219	235	µmhos/cm
Turbidity	26.04.2006	10.6	26.7	320	J.T.U
DO	26.04.2006	5.4	5.2	4.3	mg O_2/L
$PO_4^{-}P$	26.04.2006	0.089	0.09	0.08	mgP/L
NH_4 - N	26.04.2006	0.06	0.063	0.095	mgN/L
$NO_3^{-}N$	26.04.2006	0.002	0.004	0.061	mgN/L

2.3.3 Mosquito Identification

The mosquito larvae were identified in the field as either anopheles or culicine larvae using the keys of Gillies and DeMeillon [40]. The identified larvae were recorder by habitat type, hour of the day and day of sampling. The immature mosquitoes were then put in plastic vessels containing water from their respective sampling sites. The contents of plastic vessels were then closed in a cool box and transported to a nearby health center (Ratuoro Health Center) for indoor breeding. The intension was to rear larvae to adult stage for mosquito identification to species level. However, some larvae drowned during transportation due to long walking distance from the sites in swamp land. From the percentage larvae that drowned, culicine larvae had higher rates of larval mortality (52%) compared to anophelines larvae (36%). Six mosquito cages measuring 30 cm length by 30 cm width and 30 height were locally made for mosquito rearing. Three cages for anopheles larvae each containing samples from one habitat for three habitats, while the other three were for culicines divided by the number of habitats. During rearing the larvae were fed on particles of biscuits, as the development of immature mosquitoes is known to benefit from sugary substances. Since the focus was on mosquito vectors of malaria parasite, only adult anopheles mosquitoes were further identified to species level using the keys of Gillies and DeMeillon [40]. The adult anopheles mosquitoes were identified morphologically with the aid of a light microscope at the center for insect physiology and ecology (ICIPE), Mbita district. The mosquito body features considered in the identification exercise included veins of wings, speckled legs and number of bands, as well as laterally projecting tufts of scale on the abdominal segments.

2.4 Statistical Analysis

Statistical analysis was done using SPSS for windows version 17.0. Descriptive measures of central tendency and spread were analyzed in terms of means, range and standard deviation. Association of immature mosquitoes' distribution and abundance with the physico-chemical variables in breeding waters (habitats) was examined by bivariate factor analysis. Mosquito larval species abundance (expressed as mean number of immatures/sample size) in different breeding habitat types was examined for significant mean differences between habitats using Post-Hoc analysis of variance (One Way ANOVA). The influence of water quality parameters such as temperature, dissolved oxygen, pH, turbidity and conductivity on the occurrence and abundance of *Anopheles larvae* and *Culine larvae* was analyzed using Pearson correlation "r" test (significance represented by ρ-value) for parametric variables. All numbers of sampling units per habitat, including those with no larvae, were included in the pupation abundance analysis.

2.5 Ethical Consideration

This study did not involve the use of human subjects for experiments and, as such, we considered the dignity of people involved at various stages of the study, as well as the integrity of the ecosystem to be of paramount importance in the research context. In this regard, research clearance to conduct this study was obtained from the Ministry of Education, Science and Technology - Kenya, prior to the commencement of field experiments. Research clearance No. MOEST 13/001/36C 37/2 was registered in the name of Nina Pius Mbuya.

3 Results

A total of 1531 mosquito larvae were sampled from the three habitats (control pond, treated pond and swamp pools) during field work. Of this number, 987 (64.5%) were from control pond, 418 (27.3%) from treated pond, while the swamp pools had the least larvae samples of 126 (8.2%). Anopheles larvae accounted for 57.7% (884), while culicine larvae accounted for 42.3% (647) of all mosquito larvae sampled from the beginning of April to the end of August 2006. Figure 1 shows distribution of adult mosquitoes against larvae population that was reared indoors, marked by respective habitat of their origin. The results summarized in figure 1 show that only 570 (65%) adult mosquitoes emerged from all larvae reared in cages indoor (877), indicating high larval mortality at 35%.

A good number of reared mosquito larvae, 360 (63.6%) out of 566 immature anopheles mosquitoes, survived to adult stage, while 210 (67.5%) out of 311 culicine larvae survived to mature stage of mosquito development. Anopheles larvae collected in clean water environment of control pond were more likely to survive to adult stage (70.02%) compared to anopheles larvae from treatment pond (19.23%) and swamp pools (11.76) respectively. The trend was similar for culine larvae, except for the treatment pond which showed high culicine larvae survival rates. For the culine mosquitoes, control pond had the highest culicine larvae survival rate at (84.16%) followed by treatment pond (60.22%) and swamp pools (20%). The probability that a sampled anopheles larvae would survive to adult stage was highest for larvae collected from control pond and lowest for larvae collected from the swamp pools. Thus, much as treatment pond contained some anopheles larvae, the results show that their chance of survival was in fact very low.

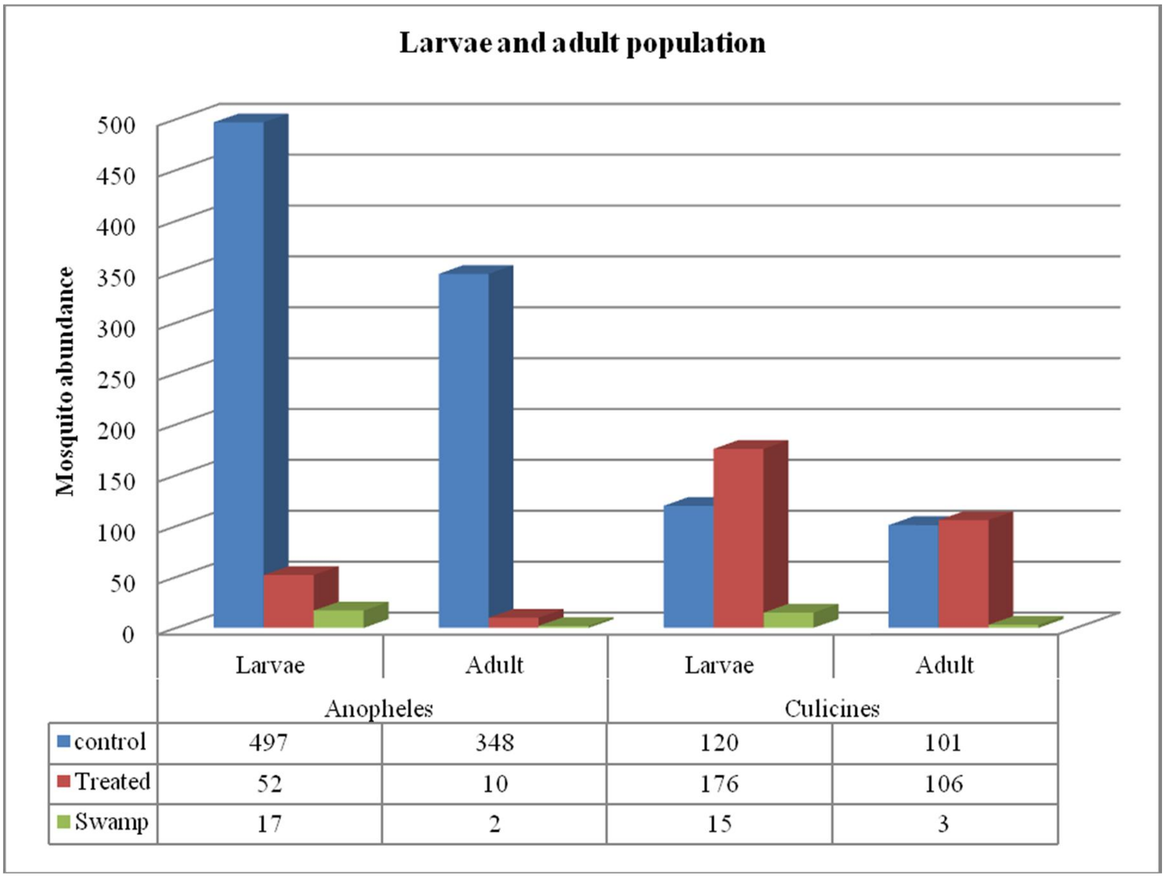

Fig. 1. Emergent adult mosquitoes from the larvae reared in cages indoor

Table 4 provides the details of physic-chemical conditions of mosquito breeding waters where anopheles and culicine larvae were collected during the study. The table 4 also shows disparities of mosquito abundance between habitats, we attribute to variations in Physico-chemical parameters tested in breeding waters that were positive for mosquito larvae. As the results indicate, conditions in control pond, which had comparatively higher concentration of dissolved oxygen and lower pH value, favored anopheles mosquitoes' breeding. On the contrary, culicine larvae occurrence in the same control pond was comparatively lower in abundance. Rather, higher abundance of culicine mosquitoes was recorded in cow-dung treated pond, which had comparatively higher level of turbidity.

Overall, the swamp pools were least preferred by both groups of mosquitoes breeding in Yala swamp wetland. The swamp pools which had the highest levels of tested water quality parameters such as Temperature, pH, conductivity and turbidity also had the lowest larvae collection. Other than the water quality parameters, biotic factors (vegetation and predators) might have played some role, by influencing habitat preference for breeding of gravid mosquitoes. However, the population mean of potential predators of mosquito larvae was higher in treated pond compared to both control pond and swamp pools. This means that non-biotic factors played significant role during the study period.

Table 4. Mean mosquito larval population by water quality parameters in each habitat

Variables	Mosquito Habitats		
	Control pond n = 40	Treated pond n = 40	Swamp pools n = 40
Anopheles larvae	20.38 ± 0.41	1.29 ± 0.23	0.41 ± 0.10
Culicine larvae	4.29 ± 0.26	9.16 ± 0.32	2.74 ± 0.29
Temp (ºC)	25.82 ± 0.59	25.93 ± 0.55	26.14 ± 0.46
pH	6.1 ± 0.18	6.27 ± 0.15	7.43 ± 0.191
Dissolved Oxygen (D.O)	5.34 ± 0.22	5.28 ± 0.19	4.34 ± 0.13
Turbidity	26.76 ± 0.34	178.63 ± 1.45	320.75 ± 1.69
Conductivity	190.5 0 ± 2.18	73.11 ± 0.64	236.15 ± 1.01

**The values are given as mean plus or minus the standard error of mean.*

Further statistical analysis of physic-chemical parameters revealed that there were significant differences in water quality between habitats during the study ($p < 0.05$), except for temperature. It was possible that the disparities in water quality between habitats created conditions which were either favorable or unfavorable to individual species of mosquitoes breeding in Yala swamp. Although the minimum and maximum ranges of selected physico-chemical characteristics of mosquito habitats did not show major variations within habitat ($p > 0.05$), significant association between mosquito abundance and specific physico-chemical parameters was detected (Table 5).

The results of Pearson correlation analysis show that anopheles larval density had strong and highly significant association with Water quality parameters such as pH, dissolved oxygen and turbidity (Table 5). Among the water quality parameters examined for mosquito breeding, only temperature was not an important factor explaining anopheles larval production in the study area. In particular, Temperature did not have significant influence on the distribution and abundance of anopheles larvae ($r=-0.01$, $P>0.05$). On the contrary, temperature was significantly associated with culine larval production in Yala swamp wetlands. The other water quality parameters important for anopheles mosquitoes' breeding (pH, Do, Turbidity and Conductivity) were not significant factors for culicines, except water pH. Table 5 shows very positive strong association between anopheles mosquito breeding and dissolved oxygen ($r = 0.677$, $p < 0.001$), and equality strong but negative significant association with water turbidity ($r = -0.854$, $p < 0.001$).

Table 5. Association of mosquito larval population with each water quality parameter

Mosquito Larvae		Water Quality Parameters				
		Temp	pH	D.O	Turbidity	Conductivity
Anopheles larvae	*Pearson correlation*	-0.010**	-0.611**	0.677**	0.854**	0.348**
	Sig.	0.915	0.001	0.001	0.001	0.001
	No. of Samples	40	40	40	40	40
Culicine larvae	*Pearson correlation*	-0.371**	-0.256**	0.163**	0.836**	0.836**
	Sig.	0.001	0.005	0.076	0.578	0.540
	No. of Samples	40	40	40	40	40
Total larvae	*Pearson correlation*	0.45**	0.762**	0.436.**	0.436.**	0.436.**
	Sig.	0.085	0.001	0.001	0.001	0.001
	No. of Samples	40	40	40	40	40

** *Pearson correlation is significant at 0.01 (Two-Tail)*

From the results of Pearson correlation analysis of the relationships between mosquito distribution and water quality parameters, it became necessary to understand if there were significant mean differences of water quality parameters between habitats. The results of Post-Hoc analysis of variance (ANOVA) of five physic-chemical parameters examined during the study, indicating mean differences of water quality between habitat (control pond, treated pond and swamp pools) are presented in table 6. The results in table 6 show that there were significant mean differences of water quality parameters between mosquito larvae habitats for the three sites ($p < 0.05$). Significant variations noted on mosquito distribution among the three habitats are reflected in the differences observed on water quality parameters tested during the study. Water quality results indicate that all parameters measured had significant variability of mean values between habitats ($p < 0.05$). Therefore, variation of mean water quality parameters between immature mosquito habitats is likely influencing anopheles mosquito breeding in control pond, treatment pond ans swamp pools.

Table 6. Mean differences of water quality parameters between mosquito lavae habitats

Variables	Habitat (I) n = 40	Mean Difference of Water Quality Parameters			
		Habitat (J) n = 40	Mean Diff (I-J)	S.E	P -value
Temperature	Control pond	Treated pond	-0.35	0.14	p = 0.012
		Swamp pools	0.3	0.14	p = 0.030
	Treated pond	Swamp pools	0.65	0.15	p = 0.001
pH	Control pond	Treated pond	-0.26	0.03	p = 0.001
		Swamp pools	-1.38	0.03	p = 0. 001
	Treated pond	Swamp pools	1.12	0.03	p < 0.001
Dissolved Oxygen	Control pond	Treated pond	0.31	0.04	p < 0.001
		Swamp pools	1.12	0.04	p < 0. 001
	Treated pond	Swamp pools	0.81	0.04	p < 0.01
Turbidity	Control pond	Treated pond	-151.3	0.29	p < 0.001
		Swamp pools	-294	0.29	p < 0. 001
	Treated pond	Swamp pools	142	0.29	p < 0.001
Conductivity	Control pond	Treated pond	58.09	22.82	p = 0.012
		Swamp pools	107.09	22.82	p < 0. 001
	Treated pond	Swamp pools	49	22.82	p = 0.034

**The Mean difference is significant at the 0.05 level. Values are given to the nearest decimal where mean difference is significant at P < 0.05 based on LDC Pos-Hoc analysis of variance (ANOVA).*

The results of initial water quality analysis suggest that there was little evidence of organic pollution with cow-dung in treatment pond. These results are presented in table 3, showing relatively low levels of ammonia (0.063 mgN/L), nitrate (0.004 mgN/L) and phosphate (0.09 mgP/L) in both control and treatment ponds compared to swamp pools. The results achieved with appropriate volume of cow-dung filtrate added into the pond water marked 'treatment pond' is evidenced by anopheles mosquitoes' less preference of treatment pond water.

Table 7 summarizes the mean differences of mosquito densities between habitats in the study area. Overall, there was high significant mean difference of mosquito density between control pond and treated pond ($p < 0.001$), control pond and swamp pools($p < 0.001$), and between treatment pond and swamp pools ($p < 0.05$). A highly significant mean difference of mosquito abundance between control pond and treatment pond shows that treatment with cow-dung had significant influence on mosquito breeding during the study period.

Similarly, the significant mean difference of larvae abundance between treatment pond and swap pools indicates that water pools in undisturbed wetland are not preferred anopheles breeding grounds. This is likely so, because both control and experimental (treatment) ponds were located in the drained part of Yala swamp, whereas swamp pools were identified within the undisturbed parts of the wetland.

Table 7. Mean mosquito population differences by habitat type

Dependent Variables	Habitat (I) n = 40	Mean Difference between Habitats			
		Habitat (J) n = 40	Mean Diff (I-J)	S.E	P -value
Anopheles larvae	Control pond	Treated pond	18.85	0.82	p < 0.01
		Swamp pools	21.4	0.81	p < 0.01
	Treated pond	Swamp pools	0.91	0 .83	p > 0.05

Culicine larvae	Control pond	Treated pond	-6.14	0.53	p < 0.001
		Swamp pools	-0.26	0.52	p > 0. 05
	Treated pond	Swamp pools	8.48	0.53	p < 0.01

**The Mean difference is significant at the 0.05 level. Values are given to the nearest decimal where mean difference is significant at P < 0.05 based on LDC Pos-Hoc analysis of variance (ANOVA).*

4 DISCUSSION

The findings of this study show that of all mosquitoes breeding in Yala swamp wetlands, in western Kenya, *Anopheles* species were the most abundant mosquitoes. Other mosquito species of culicine origin only occurred in very small numbers in the three habitat categories investigated during the study. In particular, *Anopheles gambiae* complex was the most dominant malaria vector followed by *Anopheles funestus*. The two groups of anopheline species of mosquito are known vectors of malaria in the Afro-tropical regions [41]. In Kenya, the pathogen causing malaria, *Plasmodium fulciparum* is most often transmitted by *Anopheles gambiae*, with *Anopheles funestus* as another major vector [42], [43], [44]. Thus, significantly higher abundance of these species of mosquitoes present a real risk of malaria transmission in the study area.

Of all mosquito larval habitats, control pond was the most productive for mosquitoes breeding in Yala swamp. Anopheles species of mosquitoes made the most use of control pond compared to other mosquito species. Second habitat with high mosquito abundance was the treatment pond, while the swamp pools had the least number of mosquitoes sampled during the study period. On the other hand, the results of biophysical conditions observed during the study show that vegetation was present in all the three habitats. Both vegetation and predators were recorded in each of the three habitats investigated for mosquito larvae during the study period. Summary of bio-physical factors (Table 2) indicate that slight variability exists between habitats, but the variability observed on vegetation and predators were fairly narrow and not statistically significant between habitats. Earlier study [45] implicates biotic factors such as vegetation types and proportion of coverage as better predictors of larval abundance than the physicochemical factors. Subsequent studies also linked the abundance of a number of mosquito specie to the presence of specific plants [46]. For instance, the observations made in previous studies revealed that dense mono-specific stands of *Typha* (cattail) with an accumulation of submerged dead stems and isolated pockets of water are suitable for mosquito breeding [47].

Although several studies have applauded significance of vegetation in mosquito habitats, including acting as larvae hide outs from predators as well as providing food materials, this study does not confirm such direct linkages. Vegetation might also create stagnant conditions by decreasing water movement, especially for the streambed breeding mosquitoes. Instead, the results of biophysical factors suggest that it was not likely that vegetation cover and predator abundance were significantly influencing mosquito larvae distribution between habitats.

On the contrary, water quality parameters varied significantly between habitats, as did dissolved oxygen (DO), pH, Turbidity and electrical conductivity (EC). There could be no doubt that the range and relative abundance of *Anopheles* species and culicine larvae are influenced differentially by ecological parameters. In this study we found no evidence that biophysical factors bear significant influence on anopheles larvae distribution in mosquito habitats investigated. Similarly, much as the effect of water quality and external factors on both the vectors and parasites of malaria are established facts, some previous studies on the ecology of malaria transmission yielded different results. As to which ecological factors are most responsible for the distribution and abundance of *Anopheles* species, significant variability between physic-chemical parameters have been observed. In this study, for instance, temperature had no significant influence on the distribution and abundance of mosquito species found in Yala swamp.

However, unlike other water quality parameters, the effect of temperature on mosquito larval development has been applauded in many studies investigating the links between water quality and mosquito species distribution and abundance [48] . In Yala swamp the mean temperature was 26 degrees centigrade during the study period. Other studies found that the optimum temperature for mosquito development is between 25 and 27 degrees centigrade [49], and the maximum temperature range for both vectors and parasites of malaria is 40 degrees centigrade. Thus it can be argued that since the mean temperature range in both wet and dry season were close to the physiological tolerance limit of malaria vectors. Accordingly, slight temperature differences among the habitat types did not therefore produce any statistically significant association with the mosquito population density in Yala swamp.

There are several published studies linking water quality parameters and mosquito abundance: In India [50], Senegal [51], Kenya [52], and in Accra, Ghana [53]. All bring out the importance of water quality parameters for *Anopheles* species breeding. Dissolved oxygen of water quality measurements showed positive and significant correlation with the distribution and abundance of mosquito vectors of malaria in Yala swamp.

Although the study findings applaud the influence physic-chemical factors have on mosquito breeding, literature on larval habitat generally demonstrate that *Anopheles* species exploit specific habitat types, with very different biological, chemical and physical characteristics. In Yala swamp, our study shows a similar trend as the abundance was greater in the control pond habitat where the conditions were optimum for anopheles larval development. However, there is scanty and inconclusive information on different water quality ranges for *Anopheles gambiae* and *Anopheles funestus* from other areas of their geographical occurrences. Thus, from the results of field experiments we observed that, of all the habitats investigated for mosquito larvae, control pond presented the most suitable habitat for *Anopheles* mosquito breeding in Yala swamp.

The treatment of pond water with cow-dung resulted in slightly acidic water with fairly clear and moderate nutrients content. These conditions are assumed to have persisted throughout the study and had the potential to influence mosquito distribution and abundance in the pond. The treatment pond was the most preferred by gravid culicine mosquitoes for their breeding compared to the other two habitats. During the study, the quantity of cow-dung material used (Table 1) resulted in significant reduction of mosquito breeding in treatment pond in general. The findings show that cow-dung polluted water was not favoured by gravid anopheles mosquitoes for breeding, yet anopheles species were the most productive group of mosquitoes in Yala swamp. The cow-dung treatment dosage ensured that material applied on experimental pond was within the range that can support diversity of important aquatic biota. While very low levels of nutrients in a local environment may affect the population and vigor of the organisms present, excess nutrients may impact negatively on desirable aquatic populations while increasing the production and growth of vector organisms. It has been established that mosquito larvae filter-feed on algae, particulate organic matter and bacteria suspended in the water [54]. In fact, previous studies showed that development of algae, and the growth efficiency of bacteria (critical components of the mosquito larvae diet) is dependent respectively on the availability of nitrates and phosphorus in the lakes [55]. Indeed past studies [56] give concentration between 0.1 and 4 mg $NO_3^{-}N\ L^{-1}$ for lakes and other natural water bodies, with most unpolluted waters having less than 1.0 mg/L mg $NO_3^{-}N\ L^{-1}$. Accordingly, the level of nitrate and phosphorus in the cow dung treated water pools was within the limits of less than 1.0 mg/L mg $NO_3^{-}N\ L^{-1}$.

Furthermore, the dissolved oxygen and turbidity showed positive and significant correlation with the distribution and abundance of mosquito vectors of malaria in Yala swamp during the study. Indeed, to be more precise, the mean value of dissolved oxygen in the treated pond was 5.23 mg/l during the study period; hence, it was a good fit for biodiversity of aquatic fauna and flora. According to APHA [57], the dissolved oxygen concentration above 5.0 mg/l is suitable for the support of diverse biota. Thus, while the level of pollution with dissolved cow dung was within environmentally acceptable limits, it significantly diminished the chances of mosquito breeding in standing water pools in the farmed parts of the swamp.

This study recognized the fact that an important gap exists in the entomological information regarding potential long term impact of cow dung on targeted species of *Anopheles* mosquitoes. However, we are also aware that development of resistance strain of vectors, if at all, is not likely to occur over night due to cow dung use. What is more, besides being cheap and readily available, cow dung is a more biodegradable compound for the environment. Accordingly, this study adds a voice to the existing views that sustainable malaria control program needs to strategically address a complex range of environmental and social determinants in a cost-effective manner.

The focus of this study was to emphasize the fact that effective control of a disease of public health concern requires mobilization of all segments of the society. Previous studies suggest that active and informed participation of the intended beneficiaries is paramount for effective vector control [58], [59]. Environmental manipulation is among the practices that have been tried in an attempt to inhibit breeding of mosquitoes of public health concerns. Accordingly, effective suppression of the population of malaria vectors requires active participation of the vulnerable communities. For instance, there is a need to eliminate virtually all breeding sites within 2 to 3 kilometers of a settlement, which is advisable because adult mosquitoes can fly long distances [60].

5 CONCLUSION

The results of this study show that *Anopheles* species were the most abundant group of mosquitoes breeding in Yala swamp, followed by culicines which occurred in significantly low densities. Control pond was the most productive for mosquitoes breeding in disturbed part of Yala swamp wetland, with anopheles species making the most use of it. Of all the anopheles mosquitoes sampled during the study, *Anopheles gambiae* complex was the most important species found almost exclusively in control pond which had the lowest turbidity and pH levels. Except for the variability of water quality parameters between habitats, there was no significant differences in mean frequency of other habitat conditions such as biotic factors. What is more, much as culicine larvae occurred in all the three habitats, overall their population was very low. A fairly higher abundance of culicine mosquitoes was recorded only in the treated pond compared to other habitats. This suggests that use of cow-dung might create suitable habitats for mosquitoes of culicine origin, thereby increasing local biting nuisance. However, a significant reduction in anopheles population has the potential to benefit public health consideration for advancing human wellness in order to achieve sustainable development.

These results may be useful in the mosquito larval habitat management for malaria control, which emphasizes pro-active involvement of local communities. As explained in the introduction section, managing larval habitats for malaria vectors' population reduction may facilitate significant decline in malaria prevalence in western Kenya. This is important, especially in the regions of Africa where evidence portrays malaria prevalence in a declining trend. Adopting a strategy which allows the local communities to participate in larval source reduction from the forefront of intervention has the potential to achieve a wider coverage. Ultimately any significant reduction of anopheles mosquitoes' population in expansive rural settings bears the potential to minimize risk of contact with mosquito vectors of malaria pathogen. In the end, reduced human-mosquito contacts would significantly result in low mosquito biting rates and eventually bring down infection with malaria causing parasites.

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