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The Effects of Ph and Temperature Parameters of Water on Abundance of Anopheles Mosquito Larvae in Different Breeding Sites of Kapiri Mposhi District of Zambia

[1]*Moses Musonda* and [2]*Alfred Matafwali Sichilima*

[1]M.PHIL-student in Entomology at The Copperbelt University, Zambia
[2]Biological Sciences The Copperbelt University, Zambia

Abstract: Malaria is a vector borne disease a major cause of morbidity and mortality in Zambia. It is transmitted by *Anopheles* mosquitoes. There is paucity of evidence on the effects of physicochemical parameters on larvae in Kapiri mposhi district of Zambia. The study aimed at assessing the effects of physicochemical parameters on *Anopheles* larvae abundance in different breeding sites. The district was divided into four zones and surveyed for dams, rivers, swamps, marshlands and temporal water ponds. Temperature and pH measurement of water were taken on a weekly basis using a multi parameter meter (explorer GLX Pasco). 450 *Anopheles* larvae were collected and 73.6% (n=331) emerged adults, sibling species were identified using quantitative Polymerase Chain Reaction (qPCR). The abundance of An. gambiae s.s was significantly the highest (70.9% n=223), An. arabiensis Paton (29.1% =92), An. funestus (0%) and 5.2% non-amplified. 31.8% of An. gambiae s.s was found in temporal water ponds with an average pH and temperature of 6.37 and 26.9°C, respectively. Temporal water ponds had 24.6% An. arabiensis Paton with temperature and pH of 26.9°C and 6.37. The dams and rivers had no Anopheles siblings. pH has a positive coefficient correlation (r=0.114 p=0.05) while temperature has negative correlation (r=-0.373, p=0.05). Therefore, pH and temperature affects the abundance of the *Anopheles* mosquito larvae in breeding sites of Kapiri Mposhi district.

Key words: Temperature · pH · Larvae · Water Kapiri Mposhi

INTRODUCTION

Malaria is a vector borne disease and a major cause of morbidity and mortality in Zambia. The *Anopheles* mosquito larvae, requires an aquatic environment, enabling its successive molting four times. Characteristically, the larvae of *Anopheles* mosquito do not have a siphon for breathing like most species, instead they lie parallel to the water surface in order to get oxygen for respiration. According to Centre for Disease Control and Prevention [1] larvae (wigglers or wrigglers) of all mosquitoes live in the water. Near the last abdominal segment in most species is a siphon or air tube that serves as a respiratory apparatus when the larva suspends vertically below the water surface. The larvae spend most of their time feeding on algae, bacteria and other microorganisms in the surface microlayer of water. The larvae occur in a wide range of habitats, but most species prefer clean and unpolluted water. Larvae of *Anopheles* mosquitoes have been found in freshwater or saltwater marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers while others are associated with small, temporary rain pools. Many species prefer habitats with a particular vegetation while others are without. Some breed in open, sun-lit pools, while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plant [2]. Physicochemical characteristics of mosquito breeding sites may have some effect on mosquito vectors oviposition, survival and spatial distribution. Physicochemical parameters such as temperature, salinity, conductivity, Total Dissolved Solids (TDS) and pH have a significant influence on the occurrence and larval abundance among mosquito species [3, 4]. High levels of conductivity may be due to the application of agricultural fertilizers, pesticides and herbicides [5] Permanent water

Corresponding Author: Alfred Matafwali Sichilima (PhD) Biological sciences The Copperbelt University, Zambia.
Tel: +260976445733; E-mail: alflima.mata@gmail.com.

and temporary water, species deposit their eggs directly on the water surface and they may hatch in one to four days depending on temperature [6]. Balancing of pH and ionic composition of the body fluids of Anopheles mosquito larvae is of great significance in homeostasis. The water pH of the breeding sites of mosquitoes has an effect on the distribution, dynamics and abundance of Anopheles mosquitoes [3]. The larval of mosquitoes have a wide range of pH tolerance as compared to other aquatic animals [7]. Climatic factors such as moisture index and temperature also have strong effects on the distribution and abundance of malaria vectors. There is paucity of knowledge about the effects of pH on the larval growth, development, distribution and abundance of Anopheles mosquitoes in different breeding sites of Kapiri Mposhi. The research work investigated the effects of temperature and pH of various breeding sites on Anopheles mosquito larvae in Kapiri Mposhi district of Zambia.

MATERIAL AND METHODS

The methodology employed by the researcher in order to achieve the set objectives and answer the research questions was Quantitative. Based on the procedure by Scott [8] a segment of one leg was placed directly into the PCR mixture for amplification and analysis. The procedure uses a mixture of five (5) 20-base oligonucleotides to identify Anopheles siblings. The qualitative methodology was used to obtain values for the variables which was subjected to statistical analysis using Statistics Package for Social Sciences (SPSS) version 21.0 for summary and interpretation. The following tools were used to collect data: Polymerase Chain Reaction machine (PCR), Dippers (350ml) WHO recommended size. Multi parameter pH meter, Sample bottles, Ethanol.

Breeding Sites: The district was divided into four zone as Kapiri North, Kapiri South, Kapiri East and Kapiri West. Each zone was surveyed for the breeding sites and identified as dams, rivers, marshlands, swamps and temporal water ponds. A total of twenty (n=20) breeding sites in the district were sampled and Fifty (n=50) water samples were collected from each zone on a weekly basis and therefore, a total of five hundred and fifty-six (n=556) water samples were collected over a period of one month from November to December, 2019. Fig 1 shows the different types of breeding sites.

Data Collection of Physicochemical Parameters: On a weekly basis physicochemical parameters (temperature and pH) were measured using explorer GLX Pasco multipara meter thermometer and metlerdon respectively was used on each breeding site from the four zones, Fig. 2 shows how temperature was measured. The meters were calibrated before use based on the manufacturers guidelines. A total of five (n=5) replicate measurements for temperature were recorded in situ while for pH water samples were taken from each breeding site to the laboratory for analysis. The frequency of samples taken depended on availability of breeding site in each zone.

Fig. 1: The different types of breeding sites

Fig. 2: Collection of physicochemical parameters (Temperature)

Fig. 3: *Anopheles* sampling from the breeding sites.

Temperature Monitoring: Weekly temperature and ten replicates from each breeding site were taken in the four zones for the study Fig. 2 above. The monthly average temperature was determined from the weekly average temperature for each zone.

pH Monitoring: Ten replicates samples taken at different points from each breeding site were recorded on a weekly basis and the average calculated from the four zones of kapiri mposhi district. Samples were taken from the available breeding sites in the four zones. The monthly average pH was determined from the weekly average pH for each zone.

***Anopheles* Mosquito Larvae Collection:** The Anopheles larvae were collected from various breeding sites in the four zones of Kapiri Mposhi. Ten (n=10) random scoops were sampled from each breeding site of the four zones and the average number of larvae obtained was calculated from for the four weeks period. The WHO standard dipper 350 ml was used in scooping of Anopheles mosquito larvae from the various breeding sites Fig. 3. *Anopheles* sp was distinguished from other species because of their behavior in water as they position their body parallel to the water surfaces while the Culex suspends their respiratory appendages at 45° to the water surfaces. A total of 450 *Anopheles* mosquito larvae were collected from the four breeding sites and reared at the insectary. The emerged adults were preserved in 70% ethanol and morphologically identified using a morphological key [9] from the four zones. *Anopheles* adults were preserved in silica gel for further molecular identification of their sibling species by quantitative Polymerase Chain Reaction (qPCR).

RESULTS AND DISCUSSION

Breeding Site Types and Frequency: The breeding sites were surveyed and water samples collected for *Anopheles* mosquito larvae and physicochemical analysis in the four zones of Kapiri Mposhi district. A total number of 33 sites were identified, sampled and classified as

Table 1: Breeding site types and frequency

Zone	Breeding site type					Frequency of breeding site
	Dam	River	Swamp	Marshland	Temporal water ponds	
Kapiri North	01	00	01	01	03	06
Kapiri South	00	01	01	01	06	09
Kapiri East	01	00	01	01	05	08
Kapiri West	00	00	01	01	08	10
Total	02	01	04	04	22	33

Table 2: Temperature and pH Parameters and abundance of *Anopheles* Larvae in Different Breeding Sites

Breeding site	Temperature (Mean±SD)	pH (Mean±SD)	*Anopheles* larvae (Mean±SD)
Dam	31.4±2.7	7.32±0.4	0.0±0.0
River	26.8±3.3	5.87±0.3	0.0±0.0
Swamp	26.6±3.4	6.69±0.9	10.8±9.8
marshlands	25.3±2.5	6.93±0.7	10.1±8.2
Temporal water pond	26.9±2.9	6.37±0.4	12.3±10.1

temporal and permanent water bodies. Permanent water bodies were classified as bodies which have water throughout the seasons these are dams, rivers, swamps and marshlands. On the other hand, temporal water ponds included furrows, shallow wells and animal hoof prints. The breeding site with the highest frequency 67% (n=22) in the study area was temporal water ponds which are water collection points formed after the rainfalls and would dry up after the rain season. Kapiri West zone had the highest temporal water ponds 36% (n=8), the other types of breeding sites are permanent water bodies. With regards to the type of breeding sites surveyed in Kapiri Mposhi district, Kapiri West had the highest number of breeding sites 10 while Kapiri North had the lowest number of 06 as shown in Table (1).

Temperature and pH Parameters and abundance of *Anopheles* Larvae in Different Breeding Sites: The breeding site with the highest mean temperature was the dam which was open and had no vegetation needed as resting site for adult mosquitoes before oviposition and also to bring down the temperature, according to Bayoh and Lindsay [10] found that no adults were emerging when larvae were reared below 18°C or above 32°C. Therefore, the results indicate that temperature ranged from 25-31°C in various breeding sites. The temperature in the dam was relatively high hence no larvae were found in these habitats although it was within the permissible range as reported by Bayoh and Lindsay. The mean temperature for the dam and the river were 31.4°C and 26.6°C respectively. The breeding site which were the most productive were temporal water ponds with an average temperature of 26.9°C, these parameters are favorable for growth and development of the larvae [11], the results concurs with that of [3, 5, 14, 18] which stipulated that the growth and development of Anopheles larvae increases with increase in temperature. The pH in various breeding sites ranged from 5.87-7.63, these parameters falls within the rage of 4-7.8 as reported by [12] and also that of Adebote etal [16]. Therefore, outside this range mosquito eggs, larvae and pupae growth are reduced and pH range below 4.5 or above 10, mortality occur [12]. These temporal water ponds were also in close proximity with human habitats which provide blood meal sources for the development of eggs in the female mosquitoes. The average number of *Anopheles* larvae collected was 12.3 for temporal water ponds, marshlands and swamps these habitats had favorable conditions for mosquitoes to breed they had an average of 10.1 and 10.8 respectively. These breeding sites were less permanent, small and resulting from human activities [14, 15] such as agriculture and infrastructural development. Table (2) shows the categorization of mean temperature and pH parameters measured in different breeding sites and the abundance of Anopheles larvae.

Molecular Analysis and Identification of Anopheles Species Sibling: A total number of 450 larvae were collected from the four zones over a month the period of study. Out of which 331 emerged adult mosquitoes of the genera Anopheles. The 331 Anopheles mosquitoes were further analyzed using qPCR as described by Gillies and de Meillon [9] to reveal the identity of the sibling species.

***Anopheles* Species Siblings in Different Breeding Sites:** During the sibling analyses for Anopheles using the quantitative Polymerase Chain Reaction (qPCR) two sibling species were profiled. Out of 331, 70.9 %

Table 3: Temperature and pH and Abundance of *Anopheles* Species Siblings in Different Breeding Sites

Breeding site	Temperature (Mean±SD)	pH (Mean±SD)	*An. arabiensis* Paton (Mean±SD)	*An. gambiae* Giles sensu stricto (Mean±SD)	*An. funestus* Giles (Mean±SD)
Dam	31.4±2.7	7.32±0.4	0.0±0.0	0.0±0.0	0.0±0.0
River	26.8±3.3	5.87±0.3	0.0±0.0	0.0±0.0	0.0±0.0
Swamp	26.6±3.4	6.69±0.9	3.7±1.0	13.4±11.3	0.0±0.0
marshlands	25.3±2.5	6.93±0.7	9.9±3.7	8.9±7.6	0.0±0.0
Temporal water pond	26.9±2.9	6.37±0.4	17.5±11.5	22.5±9.1	0.0±0.0

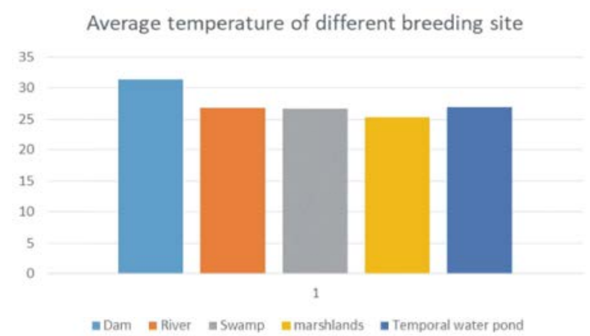

Fig. 4: Average Temperature of Different Breeding Site

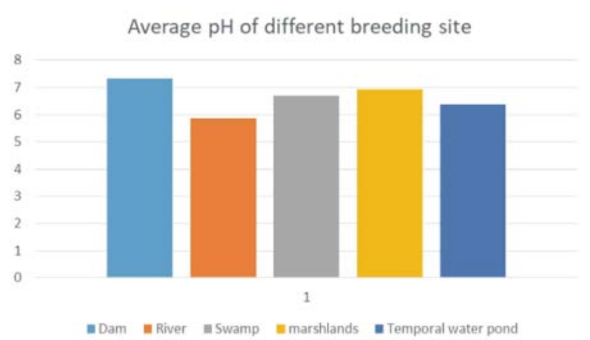

Fig. 5: Average pH of Different Breeding Sites

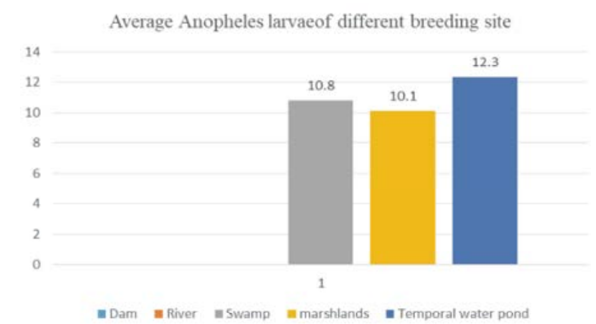

Fig. 6: Average *Anopheles* Larvae in Different Breeding Sites

comprised *An. gambiae* s.s (n=223), 24.8% (n=92) were *An. arabiensis* Paton and 5.2% (n=16) accounted for none amplified profiles. The PCR results indicates that the various breeding sites sampled had no *An. Funestus* siblings breeding in these habitats. The breeding site with the most abundant *Anopheles* species (*An. gambiae* s.s and *An. arabiensis* Paton) is the temporal water ponds with temperature and pH of 26.9°C and 6.37 respectively followed by the swamps. This result is in tandem with a report done by Dejenie and others [13] in Ethiopia that pH is associated with increase and densities of *Anopheles* larvae in temporal water ponds. There were no *Anopheles* larvae collected from both the river and the dam due to unfavorable physicochemical parameters in these breeding sites (Table 3). *An. gambiae* s.s is anthropophilic in nature hence being found in areas which are near to human habitations. The distribution of the species of *Anopheles* siblings i.e. *An. gambiae* s.s was highest in Kapiri West Zone which had 8 temporal breeding sites sampled and were near human and animal habitations. Therefore, temporal water ponds can be considered as the most dominant breeding site in kapiri mposhi district.

Various breeding sites in kapiri mposhi were surveyed and the temperature was recorded insitu over a period of one month. The dam recorded the highest temperature with an average temperature of 31.4°C as shown below in Fig. 3. The high temperature can be attributed to lack of vegetation around the dam hence not favorable for the breeding and development of the Anopheles larvae.

The dam recorded an average pH of 7.32 which is relatively neutral while the rest of the breeding sites had an acidic pH. The temporal water ponds and the river had a more acidic water than marshlands and swamps. These results of physicochemical parameters in various breeding sites concurs with that of Adebote and others [17].

Temporal water ponds had the highest average number of Anopheles larvae abundance (12.3) as shown in Fig 6 below, this is due to favorable aquatic temperature and pH which favor the development of *Anopheles* larvae. The temporal water ponds were used for agriculture activities which leads to the acidic nature of water around these breeding sites. The swamps and marshlands had 10.8 and 10.1 on average respectively.

The abundance of Anopheles larvae was the highest in the temporal water ponds followed by the swamps. These breeding sites seemed to favor the growth and

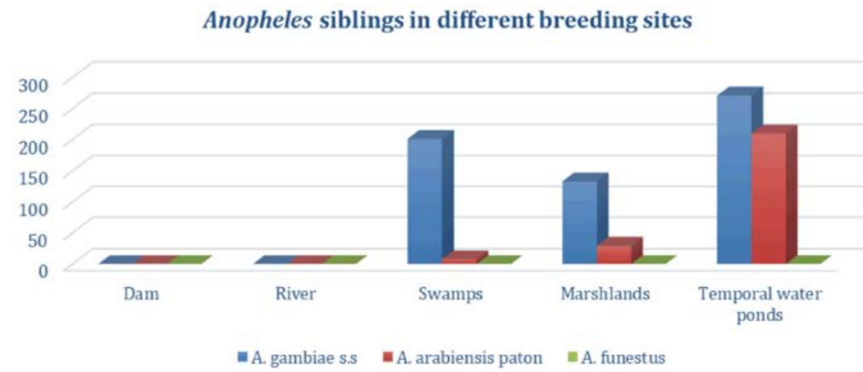

Fig. 7: *Anopheles* Siblings

Table 4: Correlations is significant p <0.05

		LARVAE	pH	TEMP
Pearson Correlation	LARVAE	1.000	0.114	-0.373
	pH	0.114	1.000	-.109
	TEMP	-0.373	-0.109	1.000
Sig. (1-tailed)	LARVAE	.	.004	.000
	pH	.004	.	.005
	TEMP	.000	.005	.
N	LARVAE	556	556	556
	pH	556	556	556
	TEMP	556	556	556

Table 5: Model Summary

Model	R	R Square	Sig. F Change
1	0.380[a]	0.145	0.000

development of the larvae due to a number of factors such as suitable temperature and pH of the water, other factors includes availability of nearby source of blood meals from animals and humans, the most abundant *Anopheles* siblings is *An. gambiae* s.s and An. Arabiensis paton which seemed to thrive better in temporal water ponds which had acidic water. It was noted that there were no *An. funestus* was found in these areas during the time of study the Fig. 7 below shows the abundance and distribution of *An. gambiae* sibling species in various breeding sites.

The research results indicate that there is a positive weakly linear correlation (r = 0.114) between the pH and the larvae abundance in the breeding sites surveyed in Kapiri Mposhi this concurs with Dejenie and others [13] in Ethiopia that pH is associated with increased densities of Anopheles larvae in temporal water ponds. While significant linear relationship existed for temperature although it's negative (r =-.373). Table 4 shows the correlations summary.

The model summary shown in Table 5 below indicates that the overall relationship between the two independent variables (temperature and pH) and dependent variable (larvae). The R value of 0.380[a] shows that a fairly weak positive linear relationship existed between independent variables and the dependent variable. This relationship is significant with a p-value for F change of 0.000.The R square value of 0.145 signifies that only 14.5% of the variation in the abundance of larvae is attributed to the variation in temperature and pH; leaving 85.5% of variation to other factors

These factors could be dissolved Oxygen, turbidity, presence or absence of predators. These factors need to be further researched on.

Based on the coefficients in Table 6 above, a multiple regression equation was formulated as follows:

$$Y=30.022+1.072X_1 -1.071X_2$$

where:

Y = Dependent variable - Larvae abundance
X_1 = Independent variable - pH
X_2 = Independent variable - Temperature
30.022 = Constant

Table 6: Coefficient

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	30.022	5.186		5.789	.000
	pH	1.072	.569	.074	1.882	.060
	TEMP	-1.071	.116	-.365	-9.219	.000

P-values for all predictor variables were generated as shown in table 6 above. The rule was to reject the null hypothesis if P-value > α (and the other way round) as it would mean the test statistic falling in the rejection region of the distribution.

CONCLUSION

- There is a relationship between the pH and Anopheles mosquito abundance in water, the pH of water which was slightly acidic hence the abundance of the larvae was more in temporal water ponds and swamps of Kapiri Mposhi district of Zambia
- Also a relationship existed between the temperature of water and Anopheles mosquito abundance in water. Higher temperatures negatively affected the abundance of Anopheles mosquito larvae because the growth and development of larvae is affected. The spatial distribution of An. gambiae s.s and An.arabiensis Paton species siblings in kapiri mposhi district have been profiled for the first time in the district.
- This research has shown for the first time that temperature and pH of water in different breeding sites of kapiri mposhi district affects the abundance of Anopheles mosquitoes however its effects is only 14.5%.
- Hence more research needs to be done to understand other factors that can affect the larvae. The research was done during the rainy season, further, research can be done during the dry season.

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