International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2017; 4(2): 111-124

Received: 16-01-2017
Accepted: 17-02-2017

Goselle O Nanjul
Department of Zoology, University of JOS, Nigeria

Okafor U Chinedu
Department of Zoology, University of JOS, Nigeria.

Ngoh Job
Department of Zoology, University of JOS, Nigeria.

Joshua S Meshach
Department of Zoology, University of JOS, Nigeria.

Wuyep P Apollos
Department of Plant Science and Technology, University of JOS, Nigeria.

Chaksda Adams
Department of Zoology, University of JOS, Nigeria

Mafuyai H Babale
Department of Zoology, University of JOS, Nigeria

Correspondence
Goselle O Nanjul
PhD, Applied Entomology and Parasitology Unit, Department of Zoology, University of JOS, Nigeria

Oviposition and Cues in Black Flies: Possible clues for boom of adult population

Goselle O Nanjul, Okafor U Chinedu, Ngoh Job, Joshua S Meshach, Wuyep P Apollos, Chaksda Adams and Mafuyai H Babale

Abstract
The population dynamics of aquatic insects in a heterogeneous environment has been attributed to their patterned spatial distribution and cues. These two factors have been reported to determine the distribution of invertebrate propagules and ultimately influence the survivorship and spatial distribution of their progeny. Aquatic insects are frequently understood to have non-selective oviposition habits, but experimental data are scarce and selective oviposition may be quite common. Previous observations indicated that water velocity current such as sequential stream seeps of shallow and fast flowing rivers and streams to large rivers, tree trunks, physico-chemical parameters, submerged objects substrates available in streams such as fallen leaves, rock surfaces, log of woods or tree roots, aquatic vegetations, mud, stones and seasonal variations and type of species were important determinants of the mass distribution of black flies and oviposition sites. We quantitatively surveyed five micro habitats on three different dates to determine the community composition and the species of invertebrates at sites and to ascertain if oviposition or survival is thriving at each successful level of collections of larvae of Assop Falls. In consideration of the instars stages that were measured, it was recorded that more late instars were collected as compared to the early instars. This indicates oviposition is at an anti-climax as compared to the survival of the larval stages. In conclusion, it could be inferred that there is a great invasion of black flies in the human community which calls for a serious concern for governmental and non-governmental organizations to increase more funding to curtail health hazards that could come from black flies bites. This study provides a framework for more sophisticated questions relating to the influence of oviposition site selection on structuring populations of microinvertebrates.

Keywords: Black flies juveniles, oviposition, cues, survival, Assop Falls

1. Introduction
Black flies are known to be important medical and veterinary group of blood sucking insects that feeds on vertebrates (birds, mammals and humans), playing a major role in running water food web [1]. They have been reported to inhabit sequential stream seeps to large rivers, substrates available in streams such as fallen leaves, rock surfaces, tree roots and mud and also breed in them [1-4] ovipositing their eggs, especially during dry season on submerged objects such as rocks, leaves, aquatic vegetation, log of wood, stones, vegetation etc. [5-9]. With an appropriate physical and chemical characteristic and also depending on temperature, the eggs which are from inception creamy white (later brown) usually hatch to the larval stage developing into seven instars stages before becoming the pupa [5-9]. A key point in understanding disease transmission in different kinds of environment which could aid in appropriate planning for an effective vector control strategies is to understand the association of the various habitat gradients with populations and community composition of black flies.
In insects which spend first part of their lifecycle in aquatic habitats, the choice of an appropriate oviposition sites has significant impact on the fitness of progeny, distribution of larvae, population dynamics, impacting offspring survival, juvenile development and growth, intra- and inter-specific interactions, predator avoidance and, ultimately, offspring phenotype and fitness and the overall maternal reproductive fitness and success [10-12], whereas for herbivorous insects, choosing a plant for oviposition could be a challenging task for female herbivors since site and plant selection are crucial for the successful development of her progeny [13, 14] and thus the need to employ external stimuli (e.g. visual and olfactory cues), their own internal physiological stimuli, and a series of environmental constraints (e.g. availability of host plants) [15-17]

which is a serious factor. For the phytophagous insects, the selection of sites for oviposition is a critical factor for the survival of the offspring of an individual since these behaviours plays an important role in host specificity, the origins of host shifts, sympatric speciation and co-evolution [18]. Moreover, as noted by Clutton-Brock [19], offspring survival is one of the most important components of lifetime reproductive success. In organisms with rudimentary forms of parental care or with no parental care, oviposition site selection is considered to have an impact on parental fitness since it often determines a great extent the chances of survival of the offspring [20].

It has been observed that in certain female species of insects for e.g *Aedes aegypti*, they exhibit the "skip oviposition" behaviour, which comprises the distribution of the eggs at several breeding sites as observed under both laboratory [21-24] and field conditions [25-28], which is probably a strategy to avoid high densities of immature forms at breeding sites where food can be limited or to minimise the risks that are associated with temporary breeding sites [29]. Importantly, this behaviour has not been reported among black flies. According to Gimnig et. al. [30], understanding the patterns of larval production from aquatic habitats is critical for understanding processes affecting adult populations.

Forearmed with the knowledge that the population dynamics of aquatic insects could be affected by spatial distribution and cues, we undertook a survey on Black flies eggs and on larvae in a habitat once reported to have a continuous decline in the number of larval species at each successive period of collections (Figure 1 from Goselle et. al.) [31]. with the aim of collecting egg masses of black flies; identifying the species of black flies immature stages after three decades of previous studies; determine species composition and abundance and to determine/measure the length of the black flies larvae as indication of cues or larval survival for accurate prediction of Black flies occurrence at the generic levels at Assop falls in Plateau state Nigeria.

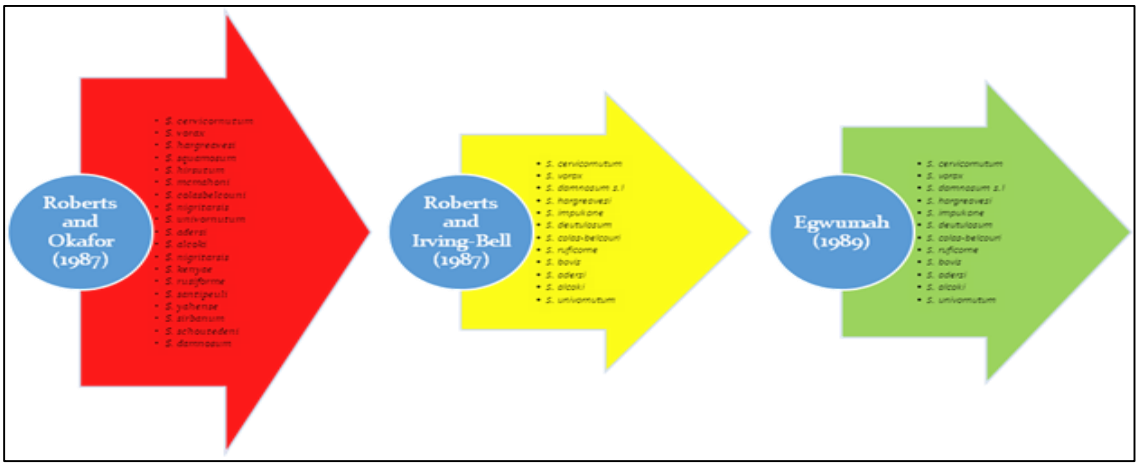

Fig 1: A schematic representation of the decline in number of the larvae species of black flies at Assop fall, Nigeria (Goselle *et. al.*, 2017)

2. Materials and Methods

2.1 Study Area

The study site was Assop fall in Hawan Kibo Village located in Riyom Local Government Area of Jos Plateau State, North Central Nigeria where previous studies by Roberts and Okafor [32] had reported nineteen species of the immature stages of black flies (Figure 1). It is located 57 km South-West of Jos, naturally endowed with a rocky bed and is a fast flowing perennial River (10 m wide), descends the western edge of Jos Plateau in Central Nigeria from a height of 1000 m to 7000 m over a distance of 4 km and projects a cool serene atmosphere. The site is a Guinea- Savannah on the slope and top of a mid-altitude of ridge of Jos Plateau beside the Jos-Kagoro road about 70km from Jos City. The area has vegetation comprising of gallery forests surrounded by grasslands. Assop River which feeds the picturesque rapids and falls drains point of the Jos Plateaus Nigeria. Its headquarters are in the town of Riyom to the north of the Area at 9°38′00″N 8°46′00″E / 9.63333°N 8.76667°E / 9.63333; 8.76667. Riyom has an area of 807 km² and a population of 131,557 as at the 2006 census, and is predominantly dominated by the Berom. The LGA has boundaries with Kaduna and Nasarawa State. It is the gateway to the State when coming from the East and from Abuja (Figure 2). Usually, two dry seasons are recorded in the area, i.e. the raining season from May to October while the dry season from October to April.

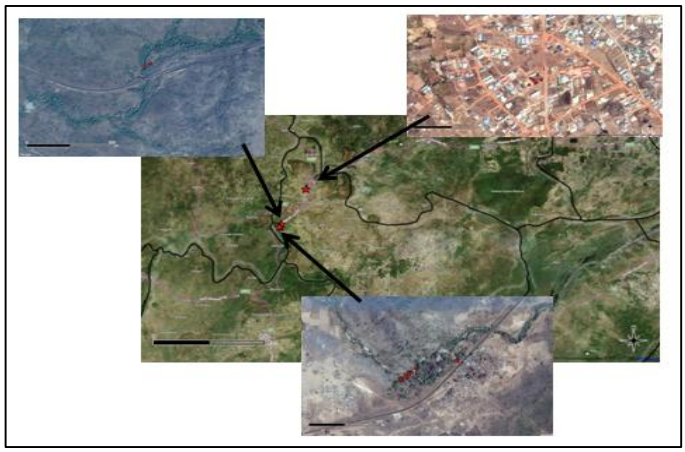

Fig 2: Map of Plateau State with Jos as headquarters with a star while the study site Assop falls has double stars, with attached top picture showing settlements surrounding Assop Falls

2.2 Duration of Study

The study was carried out on three different dates within three (3) different months i.e. from November 2015 to January 2016. The dates were staggered in other to ensure the period of breeding and emergence of new larval species is completed and

most importantly to ascertain the abundance of larvae which would enable the relating of their output to the adult stage. A Global Positioning System (GPS) was used to mark each micro-niches for collection of juveniles.

2.3 Oviposition sites, collection of eggs and counting of eggs
From the different areas and breeding sites white matrix places observed were combed for eggs and the Eggs were found to be covered by an external filamentous or lamellar matrix (exochorion) that promotes adhesion to the substrate, protects the eggs, and provides food for newly hatched larvae [7, 33]. Beneath the matrix is a waxy layer that prevents desiccation, followed by a tough envelope, the endochorion [33]. The matrix were then collected, preserved and counted according to the method described by Goldie (1982).

2.4 Sampling of larvae and measurements of water temperature
Based on phenology of the insects, sampling was conducted when the water level was low enough to allow safe access to the larvae. Five different points or micro niches were demarcated (Figure 2) and the Larvae were collected from all the available submerged substrates in the river such as fallen leaves, rock surfaces, mud and trailing grasses submerged in the river. 45 minutes exactly were spent at each area of collection sites with the depth of the river at each area recorded. The collection of larvae was done using a stainless steel plated entomological forceps. They were detached from the vegetation and placed into five separate conical flasks containing a freshly prepared carnoys fixative used for preservation of the larvae. After collecting enough larvae in each conical flask from the five different sites, they five conical flasks were transferred into a cool box containing some ice pads. This was to provide a good condition for effective preservation of the larvae.
The depths of the five micro niches were recorded. The boxes containing the collected samples were then transported to the University of Jos Undergraduate Laboratory, were they were removed from the box and 10 different Petri dishes were used in the separation of larvae and pupae for easy identification under the microscope. The same procedure was adopted for each of the dates of collections i.e. from the months of November 2015 to January 2016. The temperature of the water body was also measured.

2.5 Laboratory Work on the Larvae
Using the key for species identification by Crosskey [34] and Freeman and de Meillon [35]. Larvae were picked from the sampling containers and placed on a glass slide with at least 4-6 larvae at a time with larvae continuously wetted using the fixative to prevent desiccation. The identification and separation of the larvae and pupae was done by separating *S. damnosum* complex from non *S. damnosum* using a dissecting microscope. *Simulium damnosum sl* differs from other species by the possession of dorsal abdominal tubercles which are triangular in shape and by a row of hooklets on the proleg [34]. The best identification of *S. damnosum sl* larvae was a *Simulium damnosum* done covering dark setae. In this way, the initial separation of *Simulium damnosum* from the other *Simuliid* was achieved.
Different head pattern and plasteron gills otherwise known as the respiratory filaments which have 2 large horizontal tubes from which 3 bifid and 3 simple inner tubes arise were used as a differentiating factor. Also, the gill lie relatively flat while all the other species which are non *damnosum* had different head pattern and all gill arrangement. Morphological characteristics of the last instar larvae (matured) were identified by using the standard key of Takaoka and Chochole [36] and Crosskey [34, 37-38]. Measurements of the various instar stages were also done with the aid of a graticule placed under microscope and confirmation with a ruler and all measurements for the larvae were estimated to the nearest whole digit using the fitted eyes piece. Thereafter, each species identified were placed into separate eppendorf tubes containing 70% ethanol.

2.6 Limitation in Sampling and Identification
Inherent problem in the sampling of fresh water habitat is that of ensuring random sampling. This is consistent with the fact that velocity alone may not account for the distribution of black fly larvae. The bed rock geology of the river, the amount of submerged natural substrate and required nutrient may limit the distribution of a particular species despite appropriate water velocity.
Morphological identification of the *Simulium* species is not without its own limitations. The use of size, head pigmentation, post-genial cleft and body markings are influenced by the larvae instars stages ecological adaptation by the particular species. These markings may be bleached by the fixative before they are carried to the laboratory for identification; which may lead to mistakes in species identification.

2.7 Precautions
Care was taken to ensure that the larvae were not damaged during the process of detachment from the tip of submerged vegetation or rock particle. Fine entomological forceps, were used to hold it at the posterior end and not the anterior part to prevent damaging of the plasteron gills while detaching them from the substrates. The larvae were always kept in a cool condition. Extra care was also taken by the researchers who wore protective clothing while sampling, care was taken while stepping on surfaces during sampling.

2.8 Data Analysis
Data analysis was analyzed using the Univariate analysis for differences between subject factors i.e. species and areas, Post hoc test for least significant difference of areas and the Multivariate analysis of variance (MANOVA, PROC GLM, SAS Institute Inc.) [39] for difference in temperature at different site and to summarize invertebrate community structure in ponds/streams across all sampling dates for all sites.

Fig 3: Pictures of various microniches/microhabitats where eggs/juveniles were collected

3. Results

3.1 Black flies oviposition sites, dates and areas

The proportion of eggs collected on various substrates base on areas, dates and breeding sites are as shown in Table 1. A univariate analysis of variance to test the significant difference of collected eggs based on areas and sites showed no significant difference (Table 2) with an adjusted r-squared values at 0.441. This prompted a Post-hoc test on individual indices and a significant difference was established based on areas (Table 3) and a high significant difference based on sites (Table 4). The estimated marginal means is as shown on Figure 4.

A univariate analysis of variance based on months and sites was also conducted (Table 5). Base on individual indices of months and sites, a significant difference was established, but based on test of between subject effects between months and sites, no significant difference was established despite an adjusted r-squared value of 0.687 (Table 5). This prompted a Post-hoc test for homogeneous subsets of months and sites (Tables 6 and 7) where a high signofocant difference was established. The estimated marginal means based on substrates and months is as shown on Figure 5.

Table 1: Propotion Of Egss on Substrates Based On Areas, Dates And Breeding Sites

Areas	Dates	BREEDING SITES				
		Leaves (fallen/ standing)	Rock surfaces	Tree trunks/roots	Log of woods	Total
Area I	20/11/15	10	3	4	8	
	4/12/15	13	7	6	10	
	4/1/16	15	10	11	14	
		38	20	21	32	111
Area II	20/11/15	8	3	4	7	
	4/12/15	12	8	10	8	
	4/1/16	16	8	4	11	
		36	19	18	26	99
Area III	20/11/15	8	5	4	7	
	4/12/15	11	6	8	10	
	4/1/16	14	8	7	10	
		33	19	19	27	98
Area IV	20/11/15	8	4	3	6	
	4/12/15	9	4	3	7	
	4/1/16	10	5	6	8	
		27	13	12	21	73
Area V	20/11/15	8	5	3	7	
	4/12/15	10	4	5	12	
	4/1/16	13	6	5	10	
		31	15	13	29	88
		165	86	83	135	469

Table 2: Univariate Analysis of Variance (Tests of Between-Subjects Effects)

Dependent Variable: Value					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	395.650[a]	19	20.824	3.451	.000
Intercept	3666.017	1	3666.017	607.627	.000
AREAS	67.233	4	16.808	2.786	.039
SITES	316.317	3	105.439	17.476	.000

AREAS * SITES	12.100	12	1.008	.167	.999
Error	241.333	40	6.033		
Total	4303.000	60			
Corrected Total	636.983	59			
a. R Squared =,621 (Adjusted R Squared =,441)					

Table 3: Post Hoc Tests: AREAS (Homogeneous Subsets)

Value			
Duncan[a,b]			
AREAS	N	Subset	
		1	2
AREA 4	12	6.0833	
AREA 5	12	7.3333	7.3333
AREA 3	12	8.1667	8.1667
AREA 2	12	8.2500	8.2500
AREA 1	12		9.2500
Sig.		.053	.087
Means for groups in homogeneous subsets are displayed. Based on observed means: The error term is Mean Square (Error) = 6.033.			
a. Uses Harmonic Mean Sample Size = 12.000.			
b. Alpha =,05.			

Table 4: SITES (Homogeneous Subsets)

Value				
Duncan[a,b]				
Sites	**N**	**Subset**		
		1	**2**	**3**
Tree Trunks/Roots	15	5.5333		
Rock Surfaces	15	5.7333		
Log Of Woods	15		9.0000	
Leaves (Fallen/ Standing)	15			11.0000
Sig.		.825	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means: The error term is Mean Square (Error) = 6.033.				
a. Uses Harmonic Mean Sample Size = 15.000.				
b. Alpha =,05.				

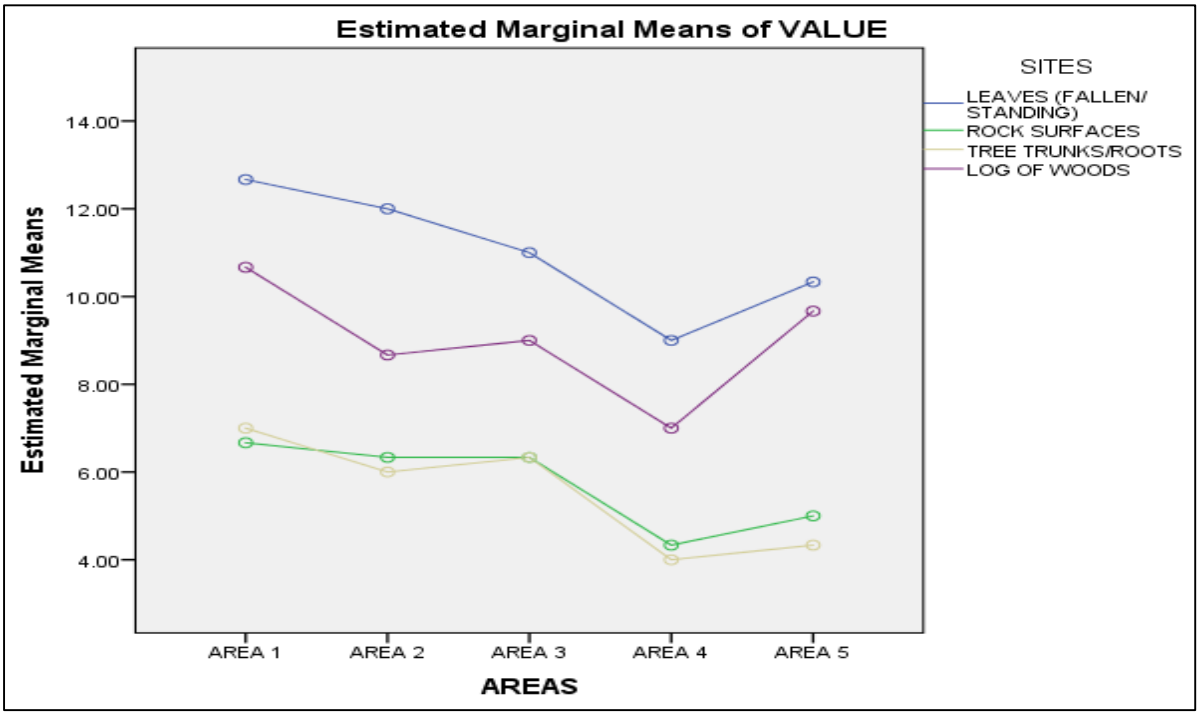

Fig 4: Estimated marginal means of eggs on substrates and breeding sites in various areas

Table 5: Univariate Analysis of Variance

Tests of Between-Subjects Effects					
Dependent Variable: Values					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	474.583[a]	11	43.144	12.752	.000
Intercept	3666.017	1	3666.017	1083.552	.000
MONTHS	147.733	2	73.867	21.833	.000
SITES	316.317	3	105.439	31.164	.000
MONTHS * SITES	10.533	6	1.756	.519	.791
Error	162.400	48	3.383		
Total	4303.000	60			
Corrected Total	636.983	59			
a. R Squared =,745 (Adjusted R Squared =,687)					

Table 6: Post Hoc Tests: MONTHS (Homogeneous Subsets)

Values				
Duncan[a,b]				
MONTHS	**N**	**Subset**		
		1	**2**	**3**
NOV	20	5.7500		
DEC	20		8.1500	
JAN	20			9.5500
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means: The error term is Mean Square(Error) = 3.383.				
a. Uses Harmonic Mean Sample Size = 20.000.				
b. Alpha =,05.				

Table 7: SITES: Homogeneous Subsets

Values				
Duncan[a,b]				
SITES	**N**	**Subset**		
		1	**2**	**3**
Tree Trunks/Roots	15	5.5333		
Rock Surfaces	15	5.7333		
Log Of Woods	15		9.0000	
Leaves (Fallen/ Standing)	15			11.0000
Sig.		.767	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on observed means: The error term is Mean Square (Error) = 3.383.				
a. Uses Harmonic Mean Sample Size = 15.000.				
b. Alpha =,05.				

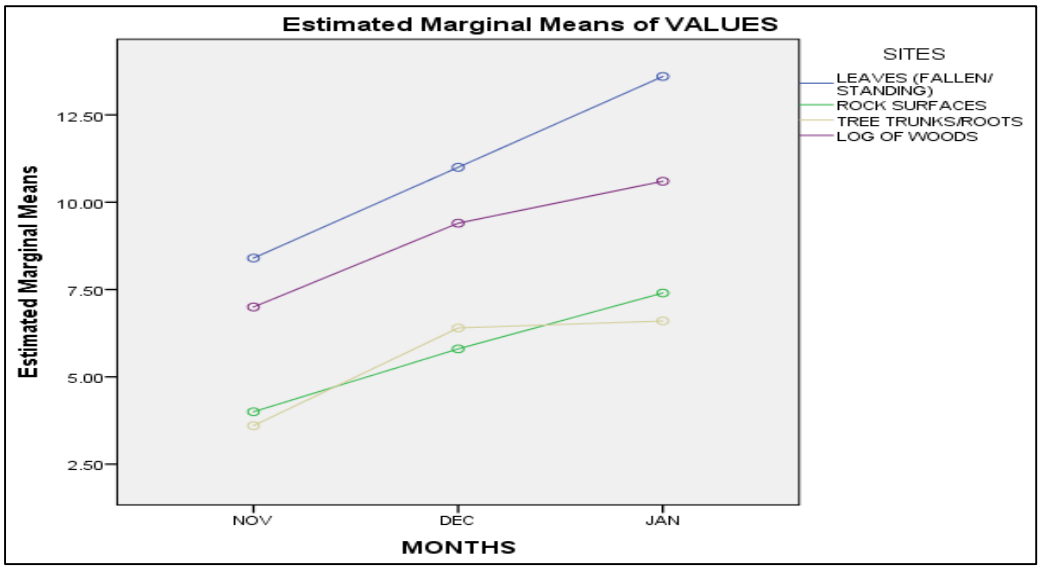

Fig 5: Estimated marginal means of eggs on substrates and breeding sites in various months

3.2 Identified Black flies larvae species with relation to sites and dates

The *Simulium* larvae species collected from the different sites of the river Assop were analyzed separately as shown in table 8. The separation of the different species was based on the difference observed on the larval stages. This table shows the different date of collection of black fly species and number of black fly species collected from the five sites. In site I, total numbers of 194 larvae were collected; site II, total number of 116 larvae were collected; site III, total number of 93 larvae were collected; site IV total of 54 larvae were collected and site V, total of 59 species were collected. The larvae collected from five sites were identified to species level to include *Simulium damnosum*, *Simulium vorax* and *Simulium hargreavesi*. *Simulium damnosum* have the highest number in sites I, III, IV and V whereas *Simulium hargreavesi was highest* in site II. Surprisingly, *S. vorax* were absent in site III.

A multivariate statistical analysis for the area with Pillar's trace, Wilk's lamda and Roy's trace all showed significant difference whereas Hotelling's trace never showed a significant difference (Table 9). Levene test for significant difference carried on the species indicates a significant difference with an adjusted R squared values of 0.490, 0.358 and -0.069 (Table 10). Estimated marginal means of the various larvae species and the dates/sites of collection are shown on Figure 6.

Table 8: Species of Black flies larvae collected from five sites with dates

Area	Date	Temp ºC	*S. damnosum*	*S. vorax*	*S. hargreavesi*	Total
Area I	20/11/15	24	40	19	39	94
	4/12/15	22	24	15	15	54
	4/1/16	16	20	9	13	42
Total		16-24	84	43	67	194
Area II	20/11/15	24	15	13	20	48
	4/12/15	22	5	8	27	40
	4/1/16	16	10	12	6	28
Total		16-24	30	33	53	116
Area III	20/11/15	24	17		14	31
	4/12/15	22	20	-	17	37
	4/1/16	16	10		15	25
Total		16-24	47	-	46	93
Area IV	20/11/15	24	11	14	-	25
	4/12/15	22		8	-	17
	4/1/16	16	96	2	4	12
Total		16-24	26	24	4	54
Area V	20/11/15	24	6	12	13	31
	4/12/15	22	8	7	6	21
	4/1/16	16	6	1	-	7
Total		16-24	20	20	19	59
G/Total		16-24	207	120	189	516

Table 9: Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	P	Partial Eta Squared
AREA	Pillai's Trace	1.392	2.1	12	30	0.043	0.46
	Wilks' Lambda	0.13	2.08	12	21.458	0.067	0.49
	Hotelling's Trace	3.244	1.8	12	20	0.118	0.52
	Roy's Largest Root	1.777	4.4	4	10	0.025	0.64

Table 10: Tests of Between-Subjects Effects

Source	Dependent Variable	Sum of Squares	df	F	P	Partial Eta Squared	Noncent. Parameter
Intercept	SPECIE 1	5529.6	1	9.45	0.012	0.5	9.5
	SPECIE 2	960.0	1	48.65	0.000	0.8	48.6
	SPECIE 3	2381.4	1	31.87	0.000	0.8	31.9
AREA	SPECIE 1	1808.4	4	0.77	0.567	0.2	3.1
	SPECIE 2	344.7	4	4.37	0.027	0.6	17.5
	SPECIE 3	882.3	4	2.95	0.075	0.5	11.8
Error	SPECIE 1	5850.0	10				
	SPECIE 2	197.3	10				
	SPECIE 3	747.3	10				

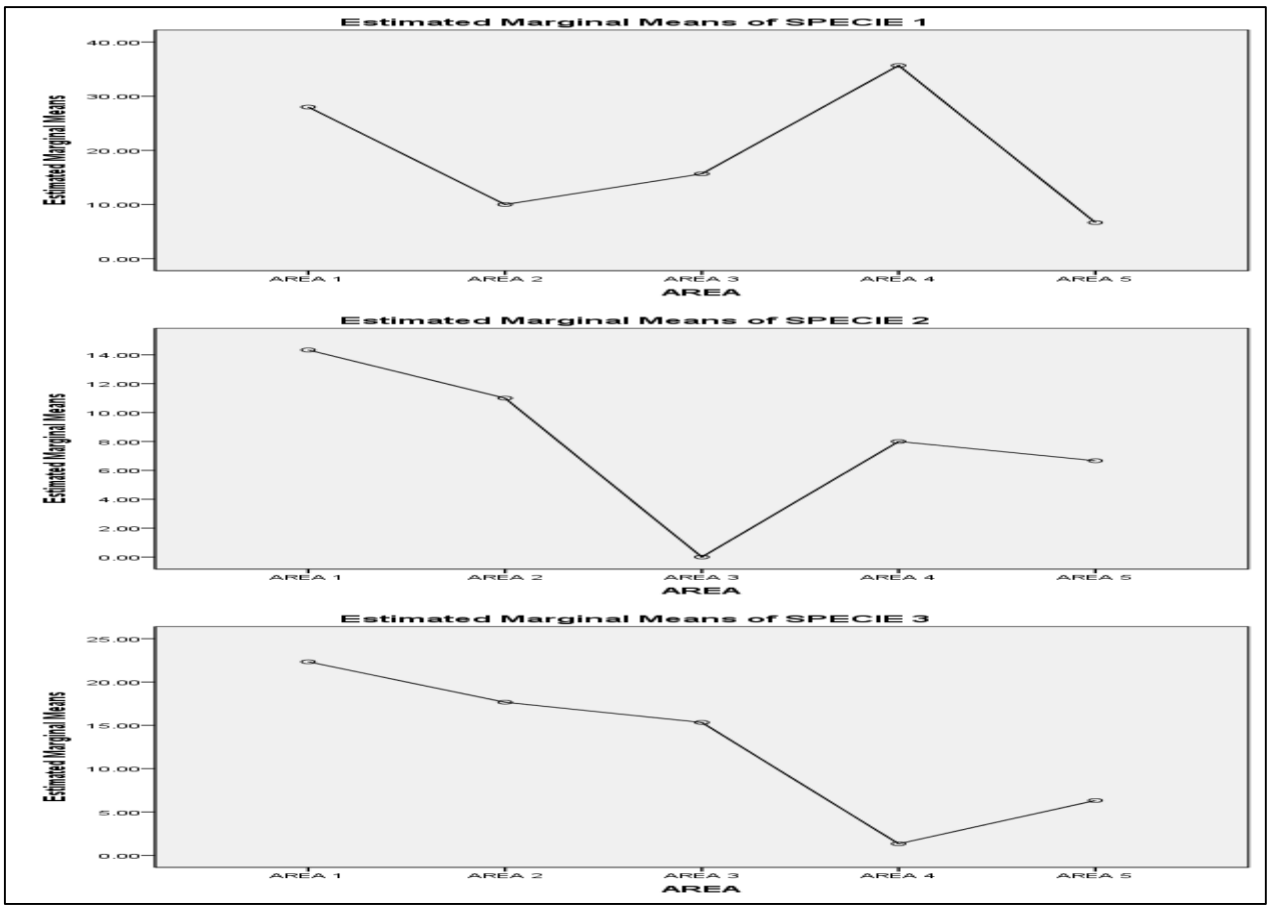

Fig 6: Estimated marginal means of the various larvae species and the dates/sites of collection

3.3 Measurements of early and late instars larval stages

In table 11, the early larval stages were assumed to be the instar stages from L1 to L3, while the late instar stages from L4 to L7. The late instar larvae stage shows high survival rate and much in number than the oviposition in all the five microniches (areas) in Assop falls. An indication that environmental factors such as temperature does not really affect the survival rate of the *Simulium* species. *Simulium hargreavesi* having higher survival rate of early and late instar larvae age in Area I, III while *S. damnosum* have high rate of larvae survival in area II, IV and V. The estimated marginal means is as shown on Figure 7.

A multivariate analysis to test between subject effects between the various instars lengths measure and species shows no significant difference (Table 12). A multiple comparison conducted showed a significant difference between the various larvae (at $P<0.001$) and especially between between *S. vorax* and other species (*hargreavesis* and *damnosum)*, but not between *S. hargreavesi* and *S. damnosum* (Table 13). This prompted a multivariate test (Table 14) based on individual instar stages and the various species where a significant difference was established between the other instars stages and those of instars stages 6 and 7 (Table 14).

Table 11: showing early and late instars larval stages collected at various micro- niches

Species	EARLY INSTAR (L1 – L3) mm						LATE INSTAR (L4 – L7) mm							(EI + LI)mm	
	L1	l2	L3	total	Mean	SD	L4	L5	L6	L7	total	Mean	SD	TOTAL	Mean
Area I No.															
S. v 43	4.75	5.45	6.3	16.5	5.5	0.63	7.15	7.85	8.35	9.1	32.45	8.12	0.71	48.95	13.61
S. h 67	5.5	6.5	7.35	19.35	6.45	0.76	8.3	9.45	10.25	11.2	39.2	9.8	1.06	58.55	16.25
S. d 84	5.95	6.1	6.85	18.9	6.3	0.39	7.8	8.8	9.65	10.55	36.8	9.2	1.02	55.7	15.5
Total 194				54.75							108.45				
Area II															
S. v 33	4.85	6.05	6.95	17.85	5.95	0.86	7.6	8.4	9.4	10.2	35.6	8.9	0.98	53.45	14.85
S. h 53	5.15	6.25	7.15	18.55	6.18	0.817	8.2	9.3	10.4	11.45	39.35	9.83	1.21	57.9	16.0
S. d 30	6.2	7.3	8.2	21.8	7.27	0.81	9.6	10.5	11.1	11.7	43.05	10.76	0.7	64.85	18.02

	5		5			7		5	5	5			9		
Total 116				58.2							118				
Area III															
S. v															
S. h 46	5.35	6.25	7.15	18.75	6.25	0.73	7.85	8.75	9.75	10.7	37.05	9.26	1.07	55.8	15.51
S. d 47	5.3	6.05	7	18.35	6.12	0.69	8	8.75	9.9	11.3	37.95	9.49	1.24	56.3	15.60
Total 93				37.1							75				
Area IV															
S. v 24	5.1	8.85	6.7	20.65	6.88	1.54	7.5	8.3	8.9	9.55	34.25	8.56	0.75	54.9	15.45
S. h 4	5.35	6.3	7.2	18.85	6.28	0.75	8.15	9.15	10.15	11.2	38.65	9.66	1.13	57.5	15.95
S. d 26	5.6	6.75	7.35	19.7	6.57	0.73	8.1	8.9	10.15	11.25	38.4	9.6	1.20	58.1	16.17
Total 54				59.2							111.3				
Area V															
S. v 20	5.15	5.85	6.65	17.65	5.88	0.61	7.45	8.1	8.8	9.55	33.9	8.48	0.78	51.55	14.36
S. h 19	5.35	6.3	7.2	18.85	6.28	0.76	8.15	8.9	10.15	11.2	38.4	9.6	1.16	57.25	15.88
S. d 20	5.5	6.4	7.25	19.15	6.38	0.71	8.2	9.1	10.05	11.1	38.45	9.61	1.07	57.6	15.99
Total 59				55.65							110.75				
G/TOTAL 516				264.90							631.95			788.40	

Based on the observed means from the Least significant difference and 95 % confidence intervals calculated, the error term mean square is 4.080 and the mean difference is significant at the 0.05 level

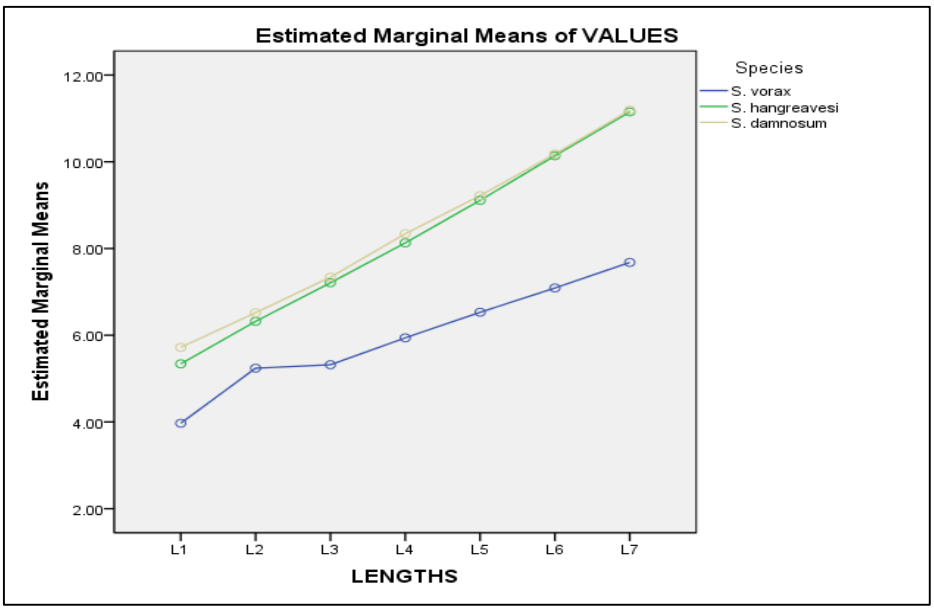

Fig 7: Estimated marginal means of lengths of larvae

Table 12: Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	P
Intercept	5919.7	1	5919.7	1450.7	0.000
LENGTHS	280.8	6	46.8	11.4	0.000
Species	125.1	2	62.5	15.3	0.000
LENGTHS * Species	13.6	12	1.1	0.2	0.991
Error	342.7	84	4.0		

Table 13: Multiple Comparisons

(I) Species	(J) Species	Mean Difference (95% Confidence interval)	P
S. vorax	*S. hangreavesi*	-2.2 (-3.2;-1.3)	<0.0001
	S. damnosum	-2.4 (-3.4;-1.4)	<0.0001
S. hangreavesi	*S. vorax*	2.2 (1.3;3.2)	<0.0001
	S. damnosum	-0.2 (-1.1;0.8)	0.743
S. damnosum	*S. vorax*	2.4 (1.4;3.4)	0.000
	S. hangreavesi	0.2 (-0.8;1.1)	0.743

Effect		Value	F	Hypothesis df	Error df	P	Partial Eta Squared
SPECIES	Pillai's Trace	1.21	1.5	14	14	0.222	0.60
	Wilks' Lambda	0.13	1.5	14	12	0.248	0.64
	Hotelling's Trace	3.94	1.4	14	10	0.297	0.66
	Roy's Largest Root	3.13	3.1	7	7	0.078	0.76

Table 14: Multivariate Tests

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	P	Partial Eta Squared	Noncent. Parameter	Observed Power
Intercept	LENGTH 1	376.5	1	376.5	221.0	<0.0001	0.9	221.0	1.0
	LENGTH 2	544.8	1	544.8	153.2	<0.0001	0.9	153.2	1.0
	LENGTH 3	658.0	1	658.0	214.5	<0.0001	0.9	214.5	1.0
	LENGTH 4	837.0	1	837.0	216.5	<0.0001	0.9	216.5	1.0
	LENGTH 5	1030.0	1	1030.0	220.4	<0.0001	0.9	220.4	1.0
	LENGTH 6	1252.2	1	1252.2	231.4	<0.0001	1.0	231.4	1.0
	LENGTH 7	1502.0	1	1502.0	239.1	<0.0001	1.0	239.1	1.0
SPECIES	LENGTH 1	8.5	2	4.2	2.5	0.125	0.3	5.0	0.4
	LENGTH 2	4.7	2	2.4	0.7	0.531	0.1	1.3	0.1
	LENGTH 3	12.8	2	6.4	2.1	0.167	0.3	4.2	0.3
	LENGTH 4	17.7	2	8.8	2.3	0.144	0.3	4.6	0.3
	LENGTH 5	23.2	2	11.6	2.5	0.126	0.3	5.0	0.4
	LENGTH 6	31.4	2	15.7	2.9	0.094	0.3	5.8	0.4
	LENGTH 7	40.6	2	20.3	3.2	0.075	0.4	6.5	0.5
Error	LENGTH 1	20.4	12	1.7					
	LENGTH 2	42.7	12	3.6					
	LENGTH 3	36.8	12	3.1					
	LENGTH 4	46.4	12	3.9					
	LENGTH 5	56.1	12	4.7					
	LENGTH 6	64.9	12	5.4					
	LENGTH 7	75.4	12	6.3					

4. Discussion

4.1 Oviposition Sites

It has been postulated that lack of information can lead to inefficient forms of immature control for e.g. the indiscriminate use of insecticide usually applied to aquatic forms which makes it difficult evaluating the efficiency of a product and environmental impacts. In this study, egg masses which cannot be specifically linked to any black fly species were collected from four different sites with the highest being the leaf, which probably have high dissolved organic matter, followed by log of woods then rock surfaces and finally tree trunks with a significant difference established at $P< 0.05$ level of significance. Several factors have been adduced to the choice of oviposition sites by female insects which may include habitat characteristics like temperature, shape, orientation and size of substrate [40-43] and humidity [44]. Importantly, black flies which are non-social insects have a conspecific aggregation which is believed to confer a variety of fitness benefits and selection of oviposition sites is crucial for survival and for minimizing egg mortality from predation [45-47]. Moreover, as put by Bunn and Hughes [48], although several species could share the same breeding sites, each could present a different life cycle duration and oviposition preference to a specific substrate, but as noted by Coupland [49, 50]; Golini and Davies [51]; Muirhead-Thompson [52], many black fly species in the genus Simulium, including members of the *Simulium damnosum* sensu lato species complex exhibit such a behavior as well, ovipositing in aggregations on a single substrate, producing clumped masses of fertile eggs.

The question of how females find a proper egg-laying site has been raised by Crosskey [8], although Greiner [53] and Crosskey[7] hypothesized that they are able to locate suitable oviposition sites visually and anemotactically. In response to such possible questions, Freedeen *et al*. [54] have reported that oviposition may vary from eggs while females fly over water or as noted by Peterson [55], the complete submergence of the fly for egg deposition on a substrate. The incubation time may range from a few days for species without egg diapauses [56] to many months for Simuliids which over-winter in that stage [57]. Higashiura [40] reported that some insects oviposit in places protected from snow cover, while Juliano *et al*. [58] noted that others avoid habitats with high probability of dessication.

Carlsson [59] reported in their findings on egg laying pattern of *S. venustum, Odagmia* and *Eusimimlium aureum* that they all

display similar patterns of oviposition behaviour laying eggs on objects at water surfaces, climbed down in search of suitable stones, sometimes depths of 20 cm. Some black fly species such as *S. vulgare* and *S. articum* are believed to deposit their eggs loosely and singly by flying low over water surfaces [54, 59]. The question has also been raised as to whether gravid flies oviposit at their natal water ways? Rothfels [60], was able to show from cytological evidence that some species of black flies do point to site-specific oviposition preferences, but this was refuted by F.F.H. (unpublished) from a preliminary cytological work on *S. venustum* and *S. verecundum* complex from the Davies Bog and North Madawaska river sites that this may not be true as the study reveal there was no any site-specific chromosomal polymorphisms for any of the species in the complex. But the debate still rages on as to how these flies are able to lay their eggs in bactches within aggregation. A possible suggestion was premised on pheromones i.e. substances produced by members of either or both sexes [61]. This has been confirmed from number of insects like the Coleoptera [61-63], Hemiptera [61, 64], Drosophilid [65-66], haematophagous Diptera [67], culicine mosquitoes [68], phlebotomine sandflies [69-70], and active components of a pheromone eliciting larval aggregation identified in the viviparous tsetse (Glossinidae) [71]. Within the Simuliidae, a number of species other than *S. damnosum* s.l. are also known to oviposit in large aggregations [49, 72-74]. The attraction of gravid black flies to a particular oviposition site is likely to depend on a combination of visual, tactile or non-volatile chemical stimuli which indicate the quality of the site, in addition to any semiochemical cues emanating from eggs already present, as is thought to occur in *S. damnosum* s.l. [75]. Pheromones involved in such attraction might not elicit species-specific responses, and it may even be that different species produce the same aggregation pheromone, as occurs in a number of *Culex* spp mosquitoes [76, 77]. Consequently, we were unable to establish if the gravid flies oviposit at their natal ways.

Regardless of the existence of site-specific genetic markers for other populations, it has been suggested that returning to the natal waterway to oviposit may be a common behavior for simuliids in general, yet there has not been an experimental test of this behavioral hypothesis until now. Either females return to the natal waterway (i.e., they exhibit site fidelity) or they do not (i.e., there is no site fidelity). Since we were unable to test the hypothesis, we could not for certain conclude either site fidelity or no site infidelity were in play to enable field experiments to distinguish between these possibilities, but from the collections of larvae from the same designated areas, we could for certain infer that these behaviours were learned during the larval stage where close proximities to where they were initially hatched could probably be thought to be safer sites to avoid flooding and predation. In addition, since in all areas more eggs were collected on leaves which have high decay of organic materials and are not control substrates, it agrees with the findings of McCall [75] that the smell of decaying organic material attracts gravid females and induces oviposition, probably because these substrates may be suitable food sources for the larvae. In conclusion, this study calls for understanding of the oviposition behaviour and structure of eggs for the 64 species of simuliids documented in Colombia [78-80] and other species in other parts of the world.

4.2 Oviposition Cues and Survival Strategy

The present study revealed that, the late instars have longer durations than the early instars which have a shorter duration. This confirmation in the recent findings is in consonance with the findings of Crosskey and Howard [80] that early instars have a shorter duration than the late instars, when the larvae reaches its final instars, it will spin itself in a cocoon out of silk, forming a pupae. The growth rate of black fly larvae depends greatly on the quality and abundance of food and on water temperature.

5. Conclusion

The collection of eggs from the various substratum and most especially on leaf which have dissolved oxygen could be a target for application of control strategies. Moreover, the reduction in the number of species caught at Assop could be as a result of changes or increase in either abiotic or biotic factor which would have been non-conducive for others species or probably just an act of the survival of the fittest that would have caused migration. Although it has been noted that *S. damnosum* and *S. sirbanum* are savannah species while others like *S. yahense, S. santipauli, S. soubrense, S. sqaumosum* are forest dwelling, and since Assop fall is in the savannah region and gradually adapting to climate change, the possibility could be for the migration of other species to suitable sites that could best support their rate of water evaporation. Lastly, the collections of more late stages could be a pointer to the population of the immediate community with black flies which could portend great danger in the transmission of onchocerciasis to the available mammals or birds.

6. Recommendation

It is recommended that further research work could be carried out in the same vicinity after some months or years to ascertain if the same species are still available, gone on migration to other areas and/or reinvasion of the area by species formally reported by Roberts and Irving-Bell [81] and Egwumah[82]. Importantly, based on the site of collection of eggs, a further study could be carried out to link the specific type of Simuliids that oviposit on a particular type of substrate.

7. Acknowledgments

We are grateful to the University of Jos Institution Base Research Fund and in particular the Tertiary Education Trust Fund (TETFund), Nigeria for the funding of the research work. The present study was conducted at the Applied Entomology and Parasitology Unit of the Department of Zoology, University of Jos, Nigeria. There were no financial or non-financial competing interests influencing the interpretation of data or presentation of information to our work. ONG conceived and designed the study and wrote the manuscript. OUC, JSM, NG and CA conducted the experiment and collection of egg masses and juveniles and measurements of juveniles. WAP and HBM aided in the laboratory differentiation of juveniles and writing of manuscript. All authors wrote, read, and approved the manuscript.

8. References

1. Adler PH, McCreadie JW. Black Flies *(Simuliidae)*. In: Mullen G, Durden LA. *Medical and Veterinary Entomology;* Academic Press. 2002, 186-202.
2. Mccreadie JW, Adler PH, Hamada N, Grillet ME. Context-dependent symbiosis between Blackfly (*Diptera:*

Simuliidae) and *Trichomycete* Fungi (*Harpellales: legeriomycetaceae*). Oikos. 2006; 108:362-370.
3. McCreadie JW, Adler PH. Variation in Larval Fitness of Black flies (Diptera: *Simuliidae*) over Heterogeneous Habitats. Aqua. Insect. 2012a; 34:143-150.
4. McGreadie JW, Adler PH. The roles of abiotic factors, dispersal and species interactions in structuring stream assamblages of Black flies (Diptera: *Simuliidae*). Aquat, Biosystt. 2012b; 8:14.
5. Adeleke MA, Mafana CF, Sam-Wobo SO, Olatunde GO, Ekpo UF, Akinwale OP. Bitting Behaviour of *Simulium damnosun* Complex and *Onchocerca volvulus* Infection along Osun River, South West, Nigeria. Par. and Vec. 2010a; 3(93):1-5.
6. Adeleke MA, Mafiana CF, Sam-Wobo SO, Olatunde GO, Akinwale OP. Morphotaxonomic studies on *Simulium damnosum* Theobald complex (Diptera: Simuliidae) along Osun River, Southwestern Nigeria. Acta Entomol. Sinica, 2010b; 53(11):1319-1324.
7. Crosskey RW. The Natural History of Black flies. John Wiley and Sons, England, 1990a.
8. Crosskey RW. Natural History of Black flies, British Museum (National History), London. John Wiley and Sons. 1990b, 711.
9. Adler PH, Currie DC, Wood DM. The black flies (*Simuliidae*) of the North America XVX, Connell University press, Ithaca, New York 2004, 941.
10. Millar JG, Chaney JD, Beehler JW, Mulla MS. Interaction of the *Culex quinquefasciatus* egg raft pheromone with a natural chemical associated with oviposition sites. J of the Ame. Mosq. Control Assoc. 1994; 10(3):374-379.
11. Resetarits WJ. Oviposition site choice and life history evolution. Am. Zool. 1996; 36:205-215.
12. Spencer M, Blaustein L, Cohen JE. Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size, Ecol., 2002; 83(3):669-679.
13. Singer MC. The definition and measurement of oviposition preference in plant-feeding insects, in J. R. Miller, and M. A. Miller (eds.). Insect–Plant Interactions Springer, New York. 1986, 65-94,
14. Mayhew PJ. Adaptive patterns of host-plant selection by phytophagous insects. Oikos, 1997; 79:417-428.
15. Visser J. Host odor perception in phytophagous insects. Annu. Rev. Entomol. 1986; 31:121-144.
16. Bernays EA, Chapman RF. Host-Plant Selection by Phytophagous Insects. Chapman and Hall, New York, 1994.
17. Badenes F, Shelton A, Nault B. Evaluating trap crops for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). J Econ. Entomol. 2004; 97:1365-1372.
18. Thompson JN, Pellmyr O. Evolution of oviposition behavior and host preference in lepidoptera. Annu. Rev. Entomol. 1991; 36:65-89.
19. Clutton-Brock TH. Reproductive success. Chicago: University of Chicago Press, 1988.
20. García-Gonzalez F, Gomendio M. Oviposition site selection and oviposition stimulation by conspecifics in the golden egg bug (*Phyllomorpha laciniata*): implications for female fitness. Behav. Ecol. Sociobiol. 2003; 53:385-92.
21. Fay RW, Perry AS. Laboratory studies of oviposition preferences of *Aedes aegypti*. Mosq. News, 1965; 25:276-281.
22. Chadee DD, Corbet PS, Greenwood JJD. Egg-laying yellow fever mosquitoes avoid sites containing eggs laid by themselves or by conspecifics. Entomol. Exp. Appl. 1990; 57:295-298.
23. Corbet PS, Chadee DD. An improved method for detecting substrate preferences shown by mosquitoes that exhibit skip oviposition. Phys. Entomol. 1993; 18:114-118.
24. Chadee DD. The diel oviposition periodicity of *Aedes aegypti* (L.) (Diptera: Culicidae) in Trinidad, West Indies: effects of forced egg retention. Bull. Entomol. Resea. 2010; 100:599-603.
25. Chadee DD, Corbet PS. Seasonal incidence and diel patterns of oviposition in the field of the mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae) in Trinidad, West Indies: a preliminary study. Ann. Trop. Med. Parasitol., 1987; 81:151-161.
26. Apostol BL, Black WCIII, Reiter P, Miller BR. Use of randomly amplified polymorphic DNA amplified by polymerase chain reaction markers to estimate the number of *Aedes aegypti* families at oviposition sites in San Juan, Puerto Rico. Am. J Trop. Med. Hyg. 1994; 51:89-97.
27. Reiter P, Amador MA, Anderson RA, Clark GG. Dispersal of *Aedes aegypti* in the urban area after blood feeding as demonstrated by rubidium-marked eggs. Am. J Trop. Med. Hyg. 1995; *52*:177-179.
28. Colton YM, Chadee DD, Severson DW. Natural skip oviposition of the mosquito *Aedes aegypti* idicated by codominant genetic markers. Med. Vet. Entomol. 2003; 17:195-204.
29. Reiter P. Oviposition, dispersal and survival in *Aedes aegypti*: implications for the efficacy of controls strategies. Vector-Borne Zoonot. Dis. 2007; 7:261-273.
30. Gimnig JE, Ombok M, Kamau I, Hawley WA. Characteristics of larval Anopheline (Diptera: Culicidae) habitats in Western Kenya. J of Med. Entomol. 2001; 38:282-288. (PubMed: 11296836).
31. Goselle ON, Joshua SM, Okafor UC, Ngoh BJ, Wuyep AP, Chaksda A *et al*. Study on population dynamics of black flies imagos. J of Entomol. and Zool. Studies, 2017; 5(1):530-539.
32. Roberts DM, Okafor BC. Microdistribution of immature black flies and the effect of water velocity and turbulence. Proceedings of Nigerian/Japan Joint-Conference Jos, 1987; M-1:164-167.
33. Goldie P. Progress toward cryopreservation of black fly eggs: *in vitro* experiments and ultrastructure observations (M. S. thesis). Cornell University, Ithaca, New York. 1982, 198.
34. Crosskey RW. A re – Classification of *Simuliids* (Diptera) of Africa and its Island. Bull. British Museum (Natural History) Entomol. Suppl. 1969; 14:194-197.
35. Freeman P, De Meillon B. *Simuliidae* of Ethopian Region. London, British Museum of Nat. History, 1953, 178-203.
36. Takaoka H, Choochole W. A list of and keys of black flies (Diptera: *Simuliidae*) in Thailand. Trop. Med. Health, 2004; 32:189-197.
37. Crosskey RW. A taxonomic study of the larvae of West African Simuliidae (Diptera: Nematocera) with comments on the morphology of the larval black fly head. Bull. British Museum (Natural History) Entomol. 1960a; 10:1-

74.
38. Crosskey RW. Distribution records of the black flies (Diptera: Simuliidae) of Nigeria and the Southern Cameroon, with a key for their identification in the pupal stage. J of the West African Sc. Assoc. 1960b; 6:27-46.
39. SAS Institute Inc: SAS/STAT 9.1, 2004. Users Guide. SAS Institute Inc; Cury, NC.
40. Higashiura Y. Survival of eggs in the gypsy moth Lymantria dispar. II. Oviposition site selection in changing environments. J Anim. Ecol. 1989; 58:413-426.
41. Canyon DV, Hii JKL, Muller R. Adaptation of *Aedes aegypti* (Diptera: Culicidae) oviposition behavior in response to humidity and diet. J Insect. Physiol. 1999; 45:959-964.
42. Reich P, Downes BJ. The distribution of aquatic invertebrate egg masses in relation to physical characteristics of oviposition sites at two Victorian upland streams. Freshwater Biol. 2003a; 48:1497-1513.
43. Reich P, Downes BJ. Experimental evidence for physical cues involved in oviposition site selection of lotic hydrobiosid caddisflies. Oecologia. 2003b; 136:465-475.
44. McCall PJ, Wilson MD, Dueben BD, Clare-Bronswoort, BM de, Heath RR. Similarity in oviposition aggregation pheromone composition within the *Simulium damnosum* (Diptera: Simuliidae) species complex. Bull. Entomol. Res. 1997; 87:609-616.
45. Prokopy RJ, Roitberg BD. Joining and avoidance behavior in nonsocial insects. Ann. Rev. of Entomol. 2001; 46:631-665. PMID: 11112182
46. Faraji F, Janssen A, Sabelis MW. The benefits of clustering eggs: the role of egg predation and larval cannibalism in a predatory mite. Oecologia, 2002; 131:20-26.
47. Damman H, Cappuccino N. Two forms of egg defense in a chrysomelid beetle: egg clumping and excrement cover. Ecol. Entomol. 1991; 16:163-167.
48. Bunn SE, Hughes JM. Dispersal and recruitment in streams:evidence from genetic studies. J North Am. Benthol. Soc. 1997; 16:338-346.
49. Coupland JB. Oviposition response of Simulium reptans (Diptera: Simuliidae) to the presence of conspecific eggs. Ecol. Entomol. 1991; 16:11-15.
50. Coupland JB. Effect of egg mass age on subsequent oviposition by Simulium reptans (Diptera: Simuliidae). J. of Med. Entomol. 1992; 29:293-295. PMID: 1495045
51. Golini VI, Davies DM. Relative response to colored substrates by ovipositing blackflies (Diptera: Simuliidae). I. Oviposition by Simulium (Simulium) verecundum Stone and Jamnback. Canadian J of Zool., 1975; 53:521-535. PMID: 1131749
52. Muirhead-Thompson RC. Communal oviposition in Simulium damnosum Theobald (Diptera, Simuliidae). Nature. 1956; 178:1279-1299.
53. Grenier P. Contribution `a l´´etude biologique des Simuliides de France. Physiol. Comp. Oecol., 1949; 1:165-330.
54. Fredeen FJH, Rempel JG, Arnason AP. Egg laying habits, overwintering stages and life cycle of *Simulium arcticum* Mall. Canadian Entomol. 1951; 83(3):73-76).
55. Peterson BV. Observations on the biology of Utah black flies (Diptera: Simuliidae). Can. Ent. 1956; 88:496-507.
56. Davies DM. The ecology and life history of blackflies (Simuliidae: Diptera) in Ontario, with a discussion of a new species. Ph.D. thesis. University of Toronto, 1949.
57. Sommerman KM, Sailer RI, Esselbaugh CO. Biology of Alaskan black flies (Simuliidae, Diptera). Ecol. Monogr., 1955; 25:345-385.
58. Juliano SA, O'Meara GF, Morrill JR, Cutwa MM. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia, 2002; 130:458-469.
59. Carlsson G. Studies on Scandinavian black flies. Opuscula Entomological Supplementum 21. Entomologiska Sallskapet, 1962.
60. Rothfels K. Cytological approaches to the study of black fly systematics and evolution. In Stock, M. W. (ed.), *Applications of Genetics and Cytology in Insect Systematics and Evolution*, University of Idaho, Moscow, 1981, 67-83.
61. Borden JH. Aggregation pheromones. *in* Kerkut G.A. and Gilbert, L.I. (*Eds*) *Comprehensive insect physiology, biochemistry and pharmacology*. Oxford, Pergamon Press. 1985; 9:257-285.
62. Faustini DL, Giese WL, Phillips JK, Burkholder WE. Aggregation pheromone of the male granary weevil, *Sitophilus granarius* (L.). J of Chemical Ecol. 1982; 8:679-687.
63. Rochat D, Malosse C, Lettere M, Ducrot P, Zagatti P, Renou M *et al*. Male-produced aggregation pheromone of the American palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera, Curculionidae): collection, identification, electrophysiological activity, and laboratory bioassay. J. of Chemical Ecol. 1991; 17:2127-2141.
64. James DG, Mori K, Aldrich JR, Oliver JE. Flight-mediated attraction of *Biprorulus bibax* Breddin (Hemiptera: Pentatomidae) to natural and synthetic aggregation pheromone. J of Chemical Ecol. 1994; 20:71-80.
65. Bartelt RJ, Schaner AM, Jackson LL. *cis*-vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. J of Chem. Ecol. 1985; 11:1747-1756.
66. Bartelt RJ, Schaner AM, Jackson LL. Aggregation pheromones in five taxa of the *Drosophila virilis* species group. Physiol. Entomol. 1986; 11:367-376.
67. McCall PJ, Cameron MM. Oviposition pheromones in insect vectors, Parasitol. Today. 1995; 11(9):352-355.
68. Laurence BR, Pickett JA. An oviposition attractant pheromone in *Culex quinquefasciatus* Say (Diptera: Culicidae). Bull. of Entomol. Research, 1985; 75:283-290.
69. El Naiem DA, Ward RD. Response of the sandfly *Lutzomyia longipalpis* to an oviposition pheromone associated with conspecific eggs. Med. and Vet. Entomol. 1991; 5:219-224.
70. Dougherty MJ, Hamilton JGC, Ward RD. Isolation of oviposition pheromone from the eggs of the sandfly *Lutzomyia longipalpis.* Med. and Vet. Entomol. 1994; 8:119-124.
71. Saini RK, Hassanali A, Andoke J, Ahuya P, Ouma WP. Identification of major components of larviposition pheromone from larvae of tsetse flies *Glossina morsitans morsitans* Westwood and Glossina morsitans centralis Machado. J of Chemical Ecol. 1996; 22:1211-1220. doi: 10.1007/BF02266961PMID: 24226080
72. Chutter FM. Notes on the biology of South African Simuliidae, particularly *Simulium (Eusimulium) nigritarse*

Coquillett. News Letter of the Limnol. Soc. of South Africa, 1972; 18:10-18.
73. Imhof JE, Smith SM. Oviposition behaviour, egg-masses and hatching response of the eggs of five Nearctic species of Simulium (Diptera: Simuliidae). Bull. Entomol. Res. Research.1979; 69(03):405–25. Doi: 10.1017/ S0007485300018939.
74. Hywel-Jones NL, Ladle M. Ovipositional behaviour of *Simulium argyreatum* and *S. variegatum* and its relationship to infection by the fungus *Erynia conica* (Entomophthoraceae). Freshwater Biol. 1986; 16:397-403.
75. McCall PJ. Oviposition pheromone in the *Simulium damnosum* complex. Med. and Vet. Entomol. 1995; 9:101-108.
76. Bruno DW, Laurence BR. The influence of the apical droplet of *Culex* egg rafts on oviposition of *Culex pipiens fatigans* (Diptera: Culicidae). J of Med. Entomol. 1979; 16:300-305.
77. Laurence BR, Pickett JA. *erythro*-6-acetoxy-5-hexadecanolide, the major component of a mosquito oviposition attractant pheromone. J of the Chemistry Society: Chemical Communication. 1982; 1163:59-60.
78. Mantilla JS, Moncada LI, Matta NE, Adler PH. Two new species of black flies (Diptera: Simuliidae) from the High Andes of Colombia. Zootaxa, 2013; 3700(3):423-34. Doi: 10.11646/zootaxa.3700.3.6.
79. Adler PH, Crosskey RW. World blackflies (Diptera:Simuliidae): a comprehensive revision of the taxonomic and geographical inventory, 2014, 122. Available in: http://www.clemson.edu/cafls/biomia/pdfs/blackflyinventory.pdf (Accessed 8 March 2014).
80. Crosskey RW, Howard TM. *A New Taxonomic and Geographical Inventory of World Black flies (Diptera: Simuliidae*). The Natural History Museum, London, 1997, 144.
81. Roberts DM, Irving-Bell RJ. Nigerian blood-fed black flies (Diptera. Simuliidae) caught in flight: relative activity and host preferences. Trop. Med. and Parasitol. 1987; 38:23-26.
82. Egwumah PO. A study of the morphology and cytotaxonomy of larvae of *Simulium* species in some river systems of Plateau State, Nigeria. MSc. Thesis University of Jos, Nigeria, 1989, 102.