THREE DECADES: A REDUCTION FROM 19 TO 3 SPECIES OF BLACK FLIES JUVENILES

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(Received on Date: 8TH August 2017 Date of Acceptance: 29th October 2017)

ABSTRACT

Anthropological and environmental influence can be contributory factors to ecological speciation within and among insects. However, identifying specific survival strategy responsible for the colonization of an environment by all comers to survival of the fittest between species, for instance high rate of fecundity, adapting/resistance to changes in the environment, or cyclic or non-cyclic life history remains challenging. Here, we report an extraordinary case where we conducted a study of an environment where the population of the generics of some Black flies species (*Simulium*) were earlier reported to be nineteen (19) species and uncovered it reduced to three (3) species over a period of three decades. Via prospective studies, we were able to understand that physical parameters such as changes in hydrogen potential of water and water velocity, oviposition cues and possibly human activities could have been agents to the reduction in population of the same type of species of insects. Identification of the available larval species collected in five micro-niches at Assop fall indicated that the three predominant larvae were *Simulium damnosum, Simulium vorax* and *Simulium hargreavesi*. Our data suggest clear signs of sympatric distribution in larvae species synchronizing with the loss of adult species and probably the ability of the few species to survive in that area despite control measures put in the past to stem the transfer of Onchocerciasis with the attack on the vector species. We propose an ecological model that generational competition could be responsible for the ability of certain species of insects to out-compete others and probably develop certain innate survival strategy to remain viable in an environment.

Keyword **:** Black flies, physico-chemical parameters, oviposition, micro-niches, systematic exclusion

No: of Tables: 11 **No: of Figures: 7** **No: of References: 51**

INTRODUCTION

Black flies belong to the family Simuliidae of the order Diptera and are known to be medical and veterinary group of blood sucking insects that pest on vertebrates (birds, mammals and humans) and also play major role in running water food web (Adler *et al.* 2004). The bites of the female adults on humans have been reported to cause excruciating pains (Takaoka & Chochole 2004) and in most instances in transmission of parasites that causes river blindness leading to blindness in over 26,000 people and visual impairment in over 500,000 (WHO 1995). The effect in communities mostly affected leads to low expectations of life, blindness, social stigma where onchodermal skin lesion is recorded and responsible for the loss of 884,000 Disability Adjusted Life Years-DALYs (WHO 1995). Present counts of the flies worldwide indicate that there are over 2,151 living species (Adler & Crosskey 2010, 2014). Black flies habitat have been reported to include sequential stream seeps to large rivers, substrates available in streams such as fallen leaves, rock surfaces, tree roots and mud (Adler & McCreadie 2002; McCreadie & Adler 2006; McCreadie & Adler 2012 a, b). As a result of their affinity to shallow and fast flowing rivers and streams, they also breed in them (Cheesbrough 1987) ovipositing their eggs especially during dry season on submerged objects such as rocks, leaves, aquatic vegetation, log of wood, stones, vegetation etc (Adeleke *et al.* 2010 a, b; Crosskey 1990; Adler *et al.* 2004). The eggs which may be around 150-500 are creamy-white, triangular in shape could change to dark brown or black within 24 hours (Crosskey 1990; Adler *et al.* 2004; Adeleke *et al.* 2010 a, b). Within 4-7 days depending on temperature (21**°C**) and appropriate physical and chemical characteristics, the eggs hatch to larvae which then develop into the seventh instar stages before becoming a pupa (Adeleke *et al.* 2010 a, b; Crosskey 1990; Adler *et al.* 2004). Although hatching of eggs has also been reported to depend on species and temperature, for instance in some species, eggs undergo a sort of diapause, warming up and hatching during spring despite earlier deposited in autumn; whereas in other species, the eggs can withstand drought since they are the resistant stage in the life cycle and hatch when conditions becomes favourable. Goddard (1993) noted that the young larvae after hatching usually attach themselves to submerged rocks and in favourable conditions they remain at hatching or in some circumstances use a silk thread to drift downstream to places with better conditions thereafter passing through six stages before reaching the pupa stage. 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.

With the aid of a posterior circlet hooks or short proley near the end of the anterior on

the larva, it anchors itself to rock surfaces, on vegetation in a tempestuous stream, trickles to mighty rivers, hot spring to glacier melt water and where they are in high density, they play major role in the ecosystem and as trophic link serving as prey to many macro invertebrates and fish predators (WHO 1992; Adler *et al.* 2004; Kazanci & Eutunc 2010; Crosskey 1987; Bassey 1998). When not consumed by predators, they are either filter-feeding or scrapping and collecting food (Hershey *et al.* 1996; Maduabum 1982; Disney 1969; Colbo & Wotton 1981). Within a period of 6-12 days, the larva spins a cocoon after which the larval life cycle is completed and the pupa emerges developing to maturity within 2-5 days but still remains in water and emerge on the surface as adults during day time depending on light, species and environmental conditions such as temperature, hydrogen potential (pH), dissolved oxygen concentration, stream width, depth of stream substrates and turbidity which contributes to their diversity and distribution, but could also be prolonged at low temperature (WHO 1992; McCreadie *et al.* 2006; Landero *et al.* 2009). According to Gimnig *et al.* (2001), understanding the patterns of larval production from aquatic habitats is critical for understanding processes affecting adult populations.

Forearmed with the knowledge that anthropological/predatorily activities in an environment cum changes in environmental conditions could contribute to the distribution and abundance of Black flies in a habitat as evidenced in the continuous reduction of the number of larvae species at each successive period of collections of immature stages at Assop falls (Figure 1), a prospective study was developed with the aim of identifying the species of black flies immature stages after three decades of previous studies; determine the effect of hydrogen potential (pH); determine water velocity that best suits their population dynamics; determine if turbulence and temperature of water affects population dynamics; determine/measure the length of the black flies larvae as indication of oviposition cues or larval survival and to ascertain if environmental factors associated with rivers is important for accurate prediction of Black flies occurrence at the generic levels at Assop Falls in Plateau state Nigeria.

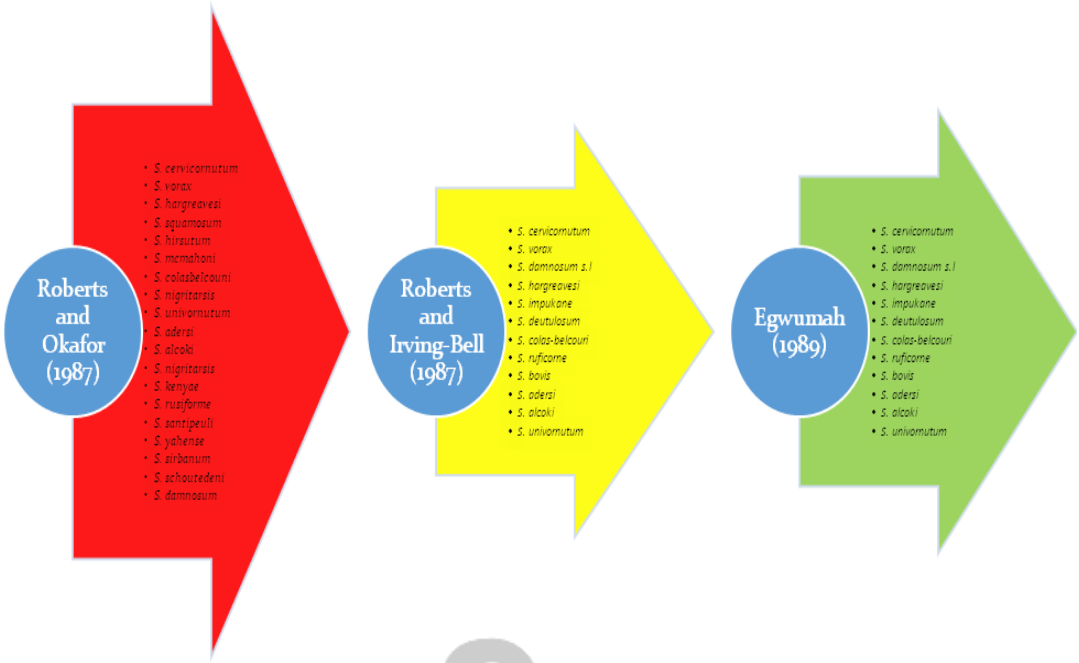


Figure 1: A schematic representation of the decline in number of the larvae species of black flies at Assop Falls, Nigeria

MATERIALS AND METHODS

Study Area

The study site was Assop Falls in Hawan Kibo Village located in Riyom Local Government Area of Jos Plateau State, North Central Nigeria where previous studies by Roberts & Okafor (1987) 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 70 km from Jos City. The area has vegetation comprising of gallery forests surrounded by grasslands. Assop Falls 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.

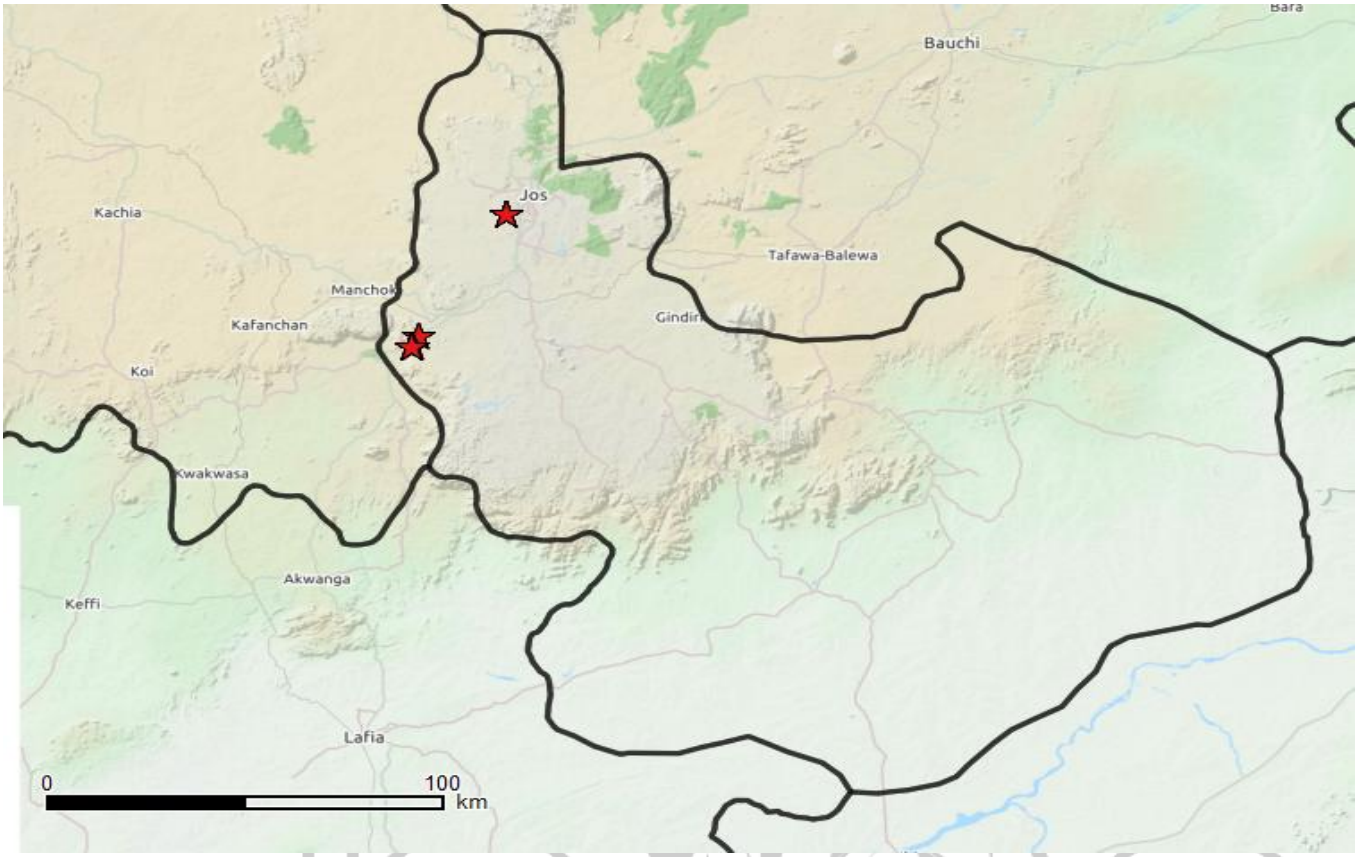


Figure 2: Map of Plateau State with Jos as headquarters with a star while the study site Assop Falls has double stars

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.

Sampling and measurements of depth of water, hydrogen potential, temperature and water velocity

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 3) and the Larvae and pupae 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. Pupae were seen scattered

between the larvae, an indication of the completion of the final instar stage and possible transformation to the adult stage. The collection of larvae and pupae 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 and pupae. After collecting enough larvae and pupae 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 and pupae.

The river velocity was estimated in the absence of a pitot tube by the float method. The time taken for a cork to cover a distance of two meter (one meter before and after an identified substrate) was determined using a stop watch. This was repeated three times, and the average distance in meter per second was then determined. The depths of the five micro niches were recorded. Also, Hydrogen potential meter was used in the measuring of the alkalinity/acidity of the water from the various sites and also the temperature.

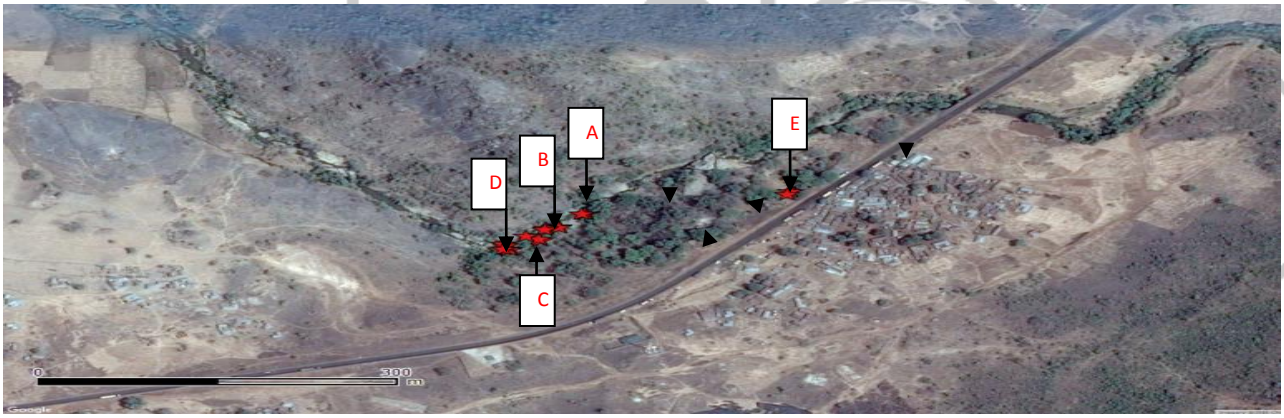


Figure 3: Pictorial view of Assop Falls with dark arrows/Letters pointing/indicating to the five micro-niches

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.

Laboratory Work on the Larvae and Pupae

Using the key for species identification by Crosskey (1969) and Freeman & de Meillon (1953). 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 (Crosskey 1969). 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 & Chochole (2004) and Crosskey (1960a, b; 1969). The larvae of black flies are brown, gray or black with light brown head, body cylindrical, somewhat club-shaped head with prominent pair of mouth brushes use for filtering food from the water. For the *Simulium vorax:* head *opotome*, evenly pigmented yellowish brown around the eye spots with some cross dark pigmentation on the cephalic apatome, postgenal clef iscordate in shape and postgenal bridge light pigmented. Abdomen gradually expands posteriorly to a rather bulbous point then abruptly contracting to posterior circlet. For the *Simulium hargreavesi gibbins*, it was observed to possess head pattern with dark infuscation which present a T-like mark postgenal clef large shape postgenal bridge shorter than the hypostonium. The larvae have a gradually expanding abdomen without ventral papillae. Thereafter, each species identified were placed into separate eppendorf tubes containing 70 % ethanol.

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 and pupae 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.

Precautions

Care was taken to ensure that the larvae and pupae 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. Also, in order to ensure best fixation of the pupae, the carnoy's fixative was always made whenever the need arose, the conical flasks containing the samples were properly closed to present the fixative from leaking. The larvae and pupae 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.

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. 2004) for difference in temperature at different site and to summarize invertebrate community structure in ponds/streams across all sampling dates for all sites.

RESULTS:

Physical parameters

Based on table 1, which shows the measurement of physical parameters, the abundance of Black flies species (Larvae and Pupae) relating to the physical parameter was observed in five different sites, it is believed that variation of the abundance of these species is caused by the variation in some parameter such as hydrogen potential which was almost neutral in some site ranging between 7.0 to 7.3. The water velocity/depth was also found to be almost the same i.e. between 1.1m/sec to 1.5m/sec and between 15.0cm to 30cm respectively. In addition, a common phenomenon was observed in the distribution of the substrates across the five sites and also the river beddings. Moreover, temperature in all niches were found to be almost the same between 16-24 ^{0}C.

Table 1: Showing the results of some physical parameters measured in the field.

	Number of *Simulium species(larvae)*	Number of *Simulium* species(pupae)	Velocity	Temp °C	Depth (cm)	pH Scale	Substrate	River Bedding
Area (I)	194	111	1.3 – 1.5m/sec	16-24	30.00	7.3	Dry leaves and Rocks	Rock and gravel bedding
Area (II)	116	115	1.1 – 1.5m/sec	16-24	26.4	7.3	Dry and fresh grasses with dry and fresh leaves	Rock and gravel bedding
Area (III)	93	87	1.2 – 1.4m/sec	16-24	24.300	7.0	Dry and fresh grasses with dry and fresh leaves	Rock and gravel bedding
Area (IV)	50	80	1.2 – 1.4m/sec	16-24	25.00	7.2	Dry and fresh leaves	Rock and gravel bedding
Area (V)	63	78	1.3 – 1.5m/sec	16-24	15.00	7.0	Dry and fresh leaves	Rock and gravel bedding
TOTAL	**516**	**471**						

Relative abundance of species at various sites

The relative abundance of larvae varies in sites I, II, III, IV and V. Overall, three species of the larvae of *Simulium* were collected in the five demarcated sites. *Simulium damnosum* has the highest prevalence, followed by *Simulium hargreavesi* and then *Simulium vorax*. A breakdown indicated that point I has the highest number of collected larvae species and the least was in point IV (Table 2). With regards to the number of larvae species and sites, the table further indicated that *Simulium damnosum* was collected the most in points I, II, IV. *S. hargreavesi* on the other hand were collected more in points II and V and never in points IV, whereas *S, vorax* were never collected in point III (Table 2). A figurative display of the collected larvae from various sites showing the estimated marginal means and the areas is displayed on Figure 4. A Univariate statistical analysis shows that there was no significant difference in the species and areas despite an adjusted R square of 0.76 (Table 3). This prompted a further Post hoc test to establish a least significant difference. Based on observed means, the error term mean square was 226.489 and a significant difference was established at 0.05 and 95 % confidence interval for the areas. A statistical analysis performed to obtain the least significant difference for the species indicates that the error term is Mean Square (Error) = 226.489 and significant difference was established between *S. damnosum* and *S. vorax*, *S. hargreavesi* and *S. vorax*, whereas no significant difference was obtained between *S. damnosum* and *S. hargreavesi* (Table 4).

Table 2: Showing relative abundance of the species of *Simulium* larvae encountered in each area (Point).

Species of Black fly	Point I	Point II	Point III	Point IV	Point V	Total
Simulium damnosum	84	30	47	26	20	207
Simulium vorax	43	33	-	24	20	120
Simulium hargreavesi	67	53	46	-	23	189
Total	**194**	**116**	**93**	**50**	**63**	**516**

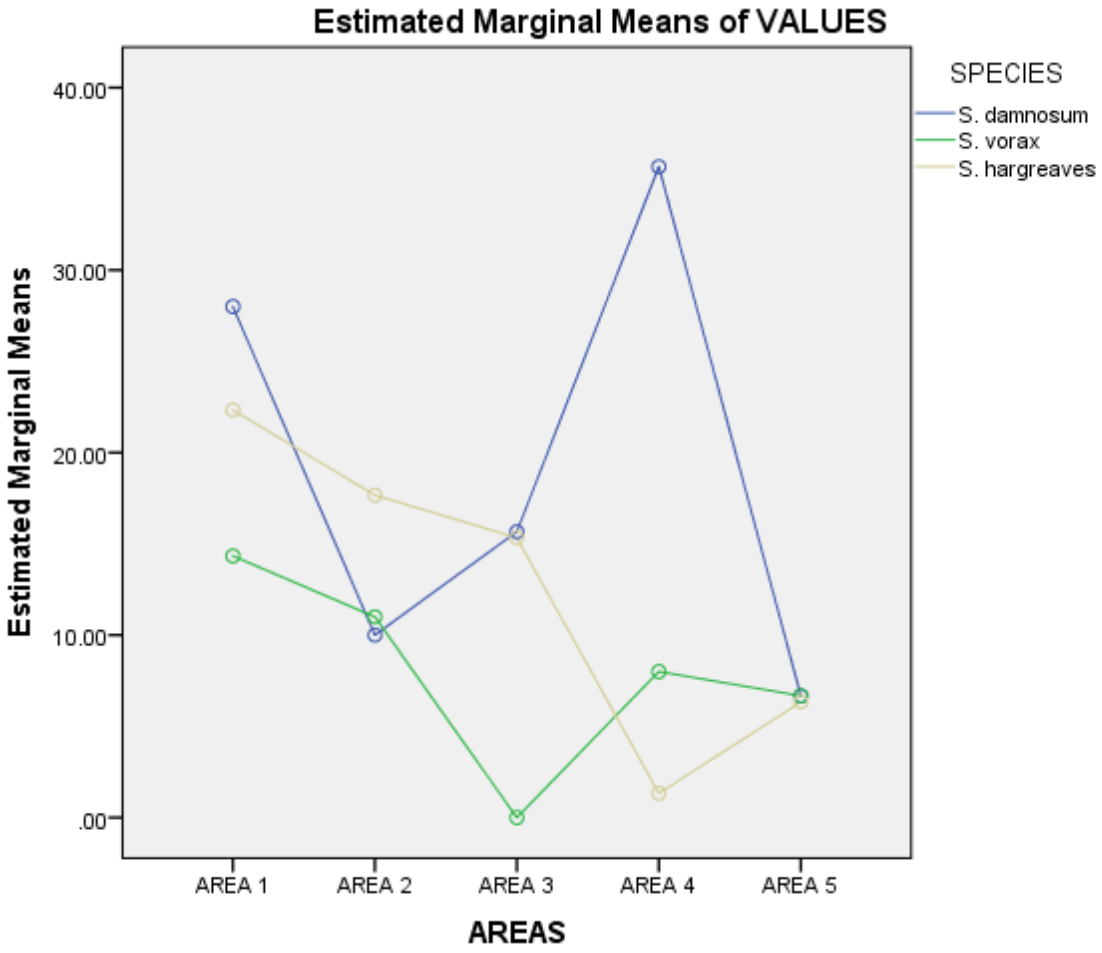


Figure 4: Estimated marginal means of collected larvae species in the various points

Table 3: Comparison of the number of individual of larva between *Simulium* species and across microniches (Area)

	Sum of Squares	df	F	P
AREAS	1129.5	4	1.2	0.313
SPECIES	950.8	2	2.1	0.140
AREAS * SPECIES	1905.9	8	1.1	0.421
Error	6794.7	30		

Table 4: Comparisons of mean number of larvae between species

(I) SPECIE	(J) SPECIES	Mean Difference (95% Confidence interval)	P
S. damnosum	*S. vorax*	11.2 (-0.02;22.42)	**0.05**
	S. hargreavesi	6.6 (-4.62;17.82)	0.239
S. vorax	*S. damnosum*	-11.2 (-22.42;0.02)	**0.05**
	S. hargreavesi	-4.6 (-15.82;6.62)	0.409

S. hargreavesi	*S. damnosum*	-6.6 (-17.82;4.62)	0.239
	S. vorax	4.6 (-6.62;15.82)	0.409

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 5. 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 6). 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 (Tables 7). Estimated marginal means of the various larvae species and the dates/sites of collection are shown on Figure 5.

Table 5: Species of Black flies larvae collected from five sites with dates

Area	Date	Temp °C	*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	96	8	-	17
	4/1/16	16		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 6: 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 7: 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				

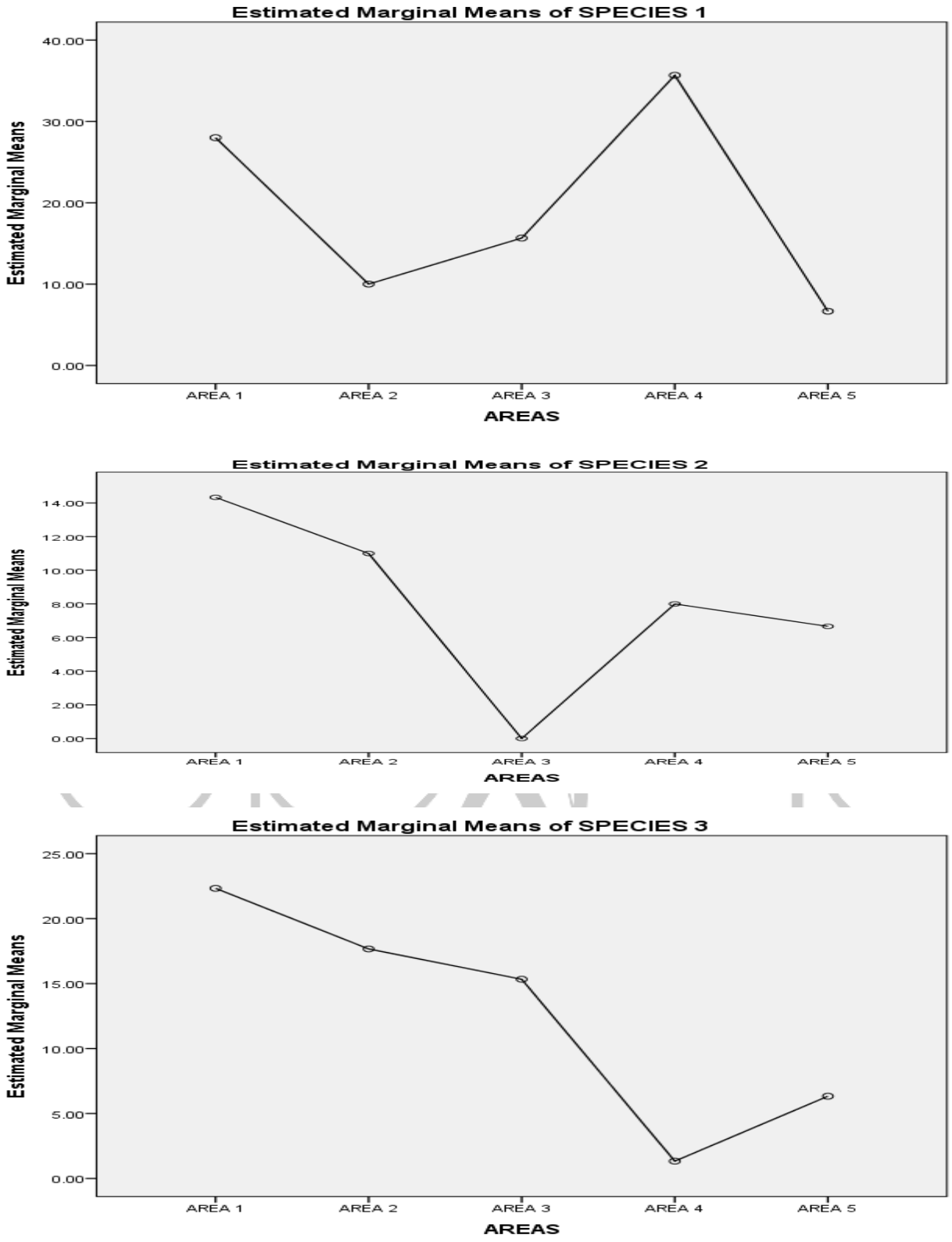


Figure 5: Estimated marginal means of the various larvae species and the dates/sites of collection

Identified Black flies pupae species with relation to sites and dates

The *Simulium* collected from the different site 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 pupae. A breakdown indicates that more of the pupae were collected in sites I as compared to the others whereas the least was on site V. A multivariate statistical conducted on the areas/sites indicates that there was no significant difference using Pillars' trace, Wilk's lamda and Hotelling's trace, whereas Roy's largest root showed a significant difference (Table 9). Levene test of statistical analysis for the pupae species indicates that the adjusted R squared values were 0.190, 0.011 and 0.056 and a significant difference was established (Tables 10). Estimated marginal means of the collected pupae from the various sites is shown on Figure 6.

Table 8: Showing these results of *Simuliids* pupae collected from the different sites

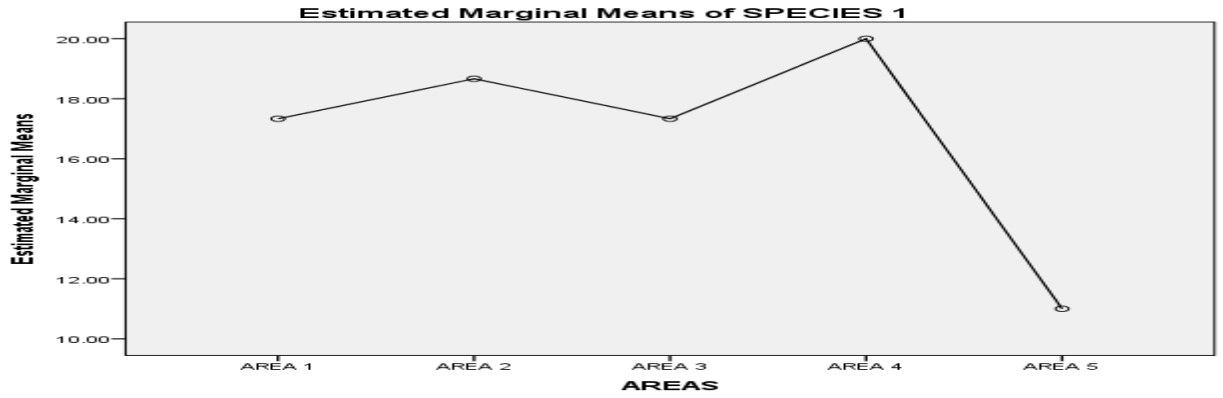
Site of collection	Date of collection	Temp °C	Total number collection	*Simulium damnosum*	*Simulium vorax*	*Simulium hargreavesi*
Site I	20/11/15	24	50	26	9	15
	4/12/15	22	36	18	11	7
	4/1/16	16	25	8	4	13
Total		**16-24**	**111**	**52**	**24**	**35**
Site II	20/11/15	24	43	22	13	8
	4/12/15	22	48	20	10	18
	4/1/16	16	24	14	3	7
Total		**16-24**	**115**	**56**	**26**	**33**
Site III	20/11/15	24	33	15	6	12
	4/11/15	22	29	19	-	10
	4/1/16	16	25	18	1	6
Total		**14-24**	**87**	**52**	**7**	**28**
Site IV	20/11/15	24	20	13	-	7
	4/11/15	22	28	21	6	1
	4/1/16	16	35	26	9	-
Total		**16-24**	**83**	**60**	**15**	**8**
Site V	20/11/15	24	30	17	3	10
	4/11/15	22	20	8	12	-
	4/1/16	16	28	8	10	10
Total		**16-24**	**78**	**33**	**25**	**20**
G/Total		**16-24**	**474**	**253**	**97**	**124**

Table 9: Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	P	Partial Eta Squared	Noncent. Parameter
AREAS	Pillai's Trace	1.10	1.4	12	30.0	0.201	0.4	17.3
	Wilks' Lambda	0.23	1.4	12	21.5	0.261	0.4	13.8
	Hotelling's Trace	2.15	1.2	12	20.0	0.351	0.4	14.3
	Roy's Largest Root	1.41	3.527[c]	4	10.0	0.048	0.6	14.1

Table 10: Tests of Between-Subjects Effects

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	P	Partial Eta Squared	Noncent. Parameter
Intercept	SPECIE 1	4267.3	1	4267.3	123.3	0.000	0.9	123.3
	SPECIE 2	627.3	1	627.3	33.7	0.000	0.8	33.7
	SPECIE 3	1025.1	1	1025.1	46.0	0.000	0.8	46.0
AREAS	SPECIE 1	143.7	4	35.9	1.0	0.434	0.3	4.2
	SPECIE 2	89.7	4	22.4	1.2	0.367	0.3	4.8
	SPECIE 3	162.3	4	40.6	1.8	0.201	0.4	7.3
Error	SPECIE 1	346.0	10	34.6				
	SPECIE 2	186.0	10	18.6				
	SPECIE 3	222.7	10	22.3				



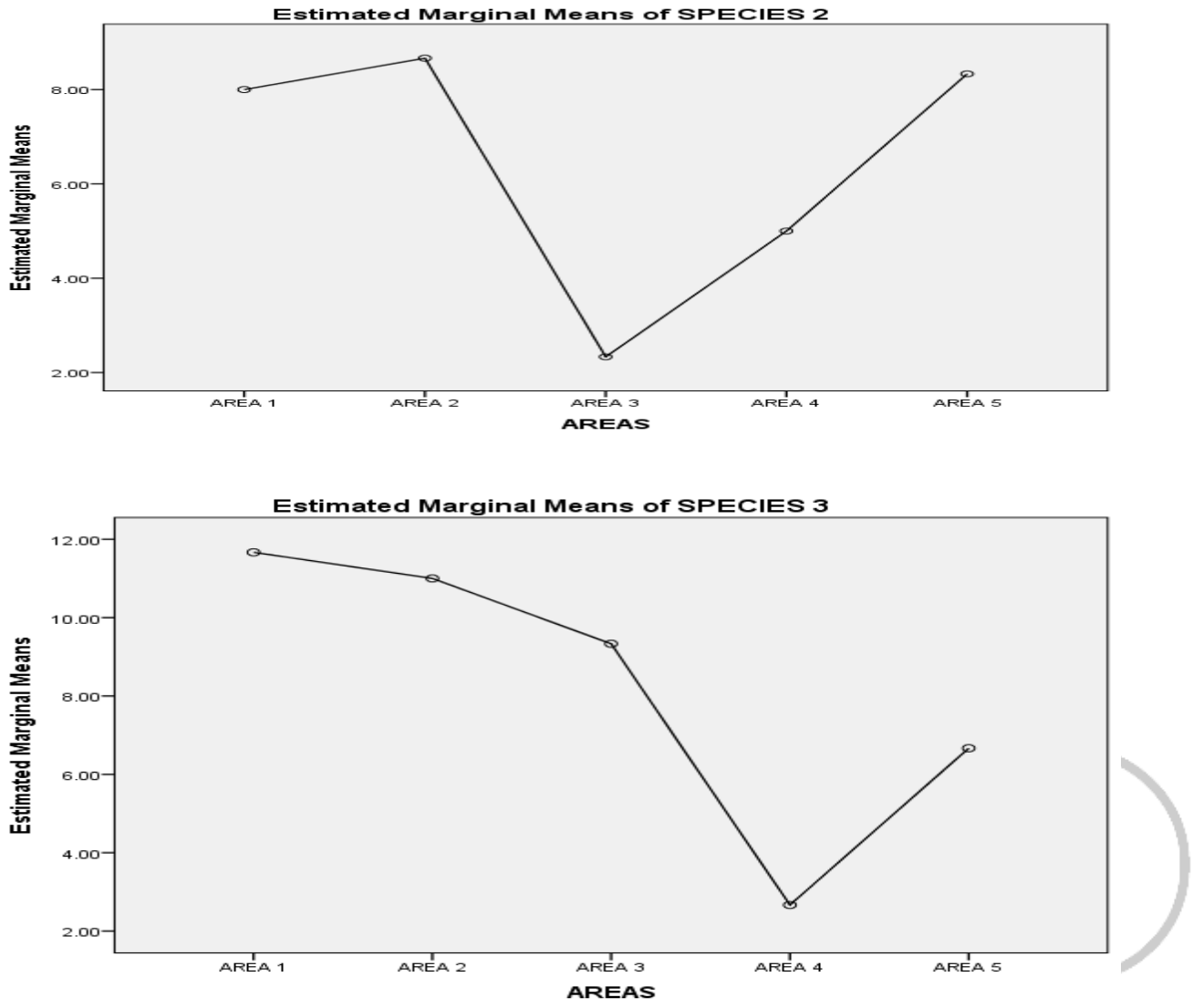


Figure 6: Estimated marginal means for Pupae of *Simulium* species collected from five sites

Prevalence of *Simulium* species at the various sites

Overall, three different group of *Simulium* species were collected. The highest were the *Edwardsellum* followed by *Metamophalus* and the least was *Medusaeform*. The percentage prevalent of the different species as obtained above from table 5 shows that there was high prevalence of *S. damnosum* in all the 5 different sites where samples were collected at Assop River with the highest peak at site IV. Next to *S. damnosum* in prevalence was *S. hargreavesi* also with highest prevalence at site III then *S. vorax* was the next to it in prevalence but with the influence of it in site V.

Table 11: Percentage of prevalence of *Simulium* species

Town	Site	Number of pupae	*Group of Simulium*	*Species of Simulium*	Total number of each species	% prevalence of species
Assop	Site I	111	*Edwardsellum*	*S. damnosum S.l*	52	47.3%
			Metomophalus	*S. hargreasvesi*	35	31.8%
			Medusaeform	*S. vorax*	24	21.8%
Assop	Site II	115	*Edwardsellum*	*S. damnosum S.l*	56	48.7%
			Metomophalus	*S. hargreasvesi*	33	28.7%
			Medusaeform	*S. vorax*	26	22.6%
Assop	Site III	87	*Edwardsellum*	*S. damnosum S.l*	52	59.8%
			Metomophalus	*S. hargreasvesi*	28	32.2%
			Medusaeform	*S. vorax*	7	8.1%
Assop	Site IV	83	*Edwardsellum*	*S. damnosum S.l*	60	72.3%
			Metomophalus	*S. hargreasvesi*	8	9.6%
			Medusaeform	*S. vorax*	15	18.1%
Assop	Site V	78	*Edwardsellum*	*S. damnosum S.l*	33	42.3%
			Metomophalus	*S. hargreasvesi*	20	25.6%
			Medusaeform	*S. vorax*	25	32.1%
		474			**474**	

DISCUSSIONS

For an effective study of black flies *(Simulium)* into the different species present in different family, several methods have been design and used. These methods which include morphological, cytological and recently molecular techniques are most effective on the immature stages and to some extent on the adult. In particular, larval morphological features, micro-morphological features of the chromosomes, the hypostonium in the different species, the shape number and arrangement of the pupal plasteron gills and also the shape of the cocoon have also provided valuable identification features for the different species for pupae. In addition, physico-chemical factor such as pH, water velocity, temperature of water and conductivity are all important when considering the whole complex and separation of the different cytospecies. The understandings of the various findings are as itemised:

Relative Abundance and sites of collection of immature stages of the Species of *Simulium*

Although this study was limited to the months of November, December and January, the result showed that the Assop Falls river is a good breeding site for 3 different species of *Simulium* and were common in the five different micro-niches. No new species was discovered different from those describe by Mafuyai *et. al.* (1996), but a remarkable decline was observed as compared to the relative abundance of the species identified and recorded from the previous findings of Roberts & Okafor (1987), Roberts & Irving-Bell (1985; 1987). Several factors from the above deductions could have contributed to the reduced numbers of black flies species at Assop falls and this variation in the relative abundance of the species identified could be as a result of seasonal changes, previous control measures on larvae, and also variation in some parameters of the water which would have affected the relative abundance of some species and this could invariably have had effects on the time taken for the egg to hatch into larva, for the larva to melt into the pupae and the pupae developing into the adult stages. As noted by Crosskey (1990) and Disney (1969), an important abiotic factor is temperature which would have affected larval eclosion, duration of development and emergence from egg to adult in *Simuliidae*. Other abiotic factors include hydrogen potential (pH) of the water, dissolved oxygen in water and anthropogenic activities such as fishing can also lead to decline in *Simulium* species

Simulium damnosum were more in number as compared to *S. vorax* and *S. hargreavesi*. This is in agreement with various studies carried out by Opoku (2000) in Ghana and Garms *et al.* (1985) who found out in their study that *S. damnosum* was more in number as compared to other species. Most importantly *S. damnosum* as reported by Disney & Boreham (1969) that *S. damnosum* complex is the most important predominant species in West Africa.

Physical parameters measured in the field (Environmental factors)

In the present study, *Simulium* species were encountered on plants leaves submerged in fast running water, dry leaves and rocks, dry and fresh leaves, rock and gravel beddings of Assop Falls river. This observation agrees with the findings of Davies (1968) and Adler & Crosskey (2014) that both larval stages of *Simulium* species often anchor themselves to submerged aquatic plants or on bark-sides, grasses and weeds that trial their leaves into the water, in irrigation canals sometimes is completely covered with black flies larvae and pupae. Crosskey (1981 a, b) reported that certain *Simulium* species such as *S. damnosum* and *S. hargreavesi* from Northern Nigeria prefer grasses and also *S. vorax* preferred similar type of vegetation. This observation is also in consonance with the findings of McCreadie & Adler (2006; 2012a) which reported that black fliers are terrestrial as adults, whereas their larvae and pupae are found in fast flowing water and after emergence, females mate and begin searching for a mammal or bird from which to obtain a blood meal, the protein form which is required for egg production. Hence Crosskey (1979) noted that the distribution of the black flies is largely dependent on the presence of swift, rocky rivers and such rivers he stated occurs in Northern Nigeria where the Precambian basement rocks are exposed.

The monthly results of the physico-chemical measurements are shown in the various Tables. With regards to the pH, this finding shows it ranges almost neutrality from 7.0-7.3. They varied largely with those described by Onyenwe *et al.* (2007) who reported between 5.55 - 5.88, that of Opoku (2000; 2006) who described the average pH range of *Simulium* breeding colony to be from 5.4 – 7.4, and also varies with previously described for *S. sirbanum* in particular or *S. damnosum* complex in general for river Eshi by Onyenwe *et al.* (2007) and Grunewald (1976). As observed by Quillévéré *et al.* (1977) in their findings in Côte d'Ivoire, they noted that the only physico-chemical factor which separated the different cytospecies throughout the whole year was pH, whereas Grunewald (1981) considered that water velocity, temperature, pH and conductivity were all important when considering the whole complex and as reported in his earlier findings Grunewald (1976), although not exceptional for the *S. damnosum* complex as a whole (*S. yahense* and *S. sanctipauli*) having been found at pH 5.1 and 5.5 for example..

The pH would have been appropriate for *S. sirbanum* which would have agreed with the reports of Quillévéré *et al.* (1977), Grunewald (1978), Grunewald (1981), Mafuyai (1992) and Bassey (1998) in Nigeria where they reported their preference for near neutrality of 6.2 – 7.9. But the report varied with that of Onyenwe *et al.* (2007) for river Eshi (5.6 – 5.9) which is unusually acidic for *S. sibanum*, although not exceptional for the *S. damnosum* complex as a whole (*S. yahense* and *S. sanctipauli*) having been found at pH 5.1 and 5.5 for example – Grunewald 1976). This variation in the pH could be attributed to change in

adaptation of *Simulium* species to change in climatic and human activities which is gradually turning the study area into a derived savannah from its original forest bio-climate. As noted by Colbo & Wotton (1982), that in examining the present day distribution of Simuliids, it must always be remembered that in most areas, the consequences of human activities are likely to result in discrepancies between those areas and others untouched by man. It is possible that human pollution in the river largely within the city renders this area and the city outskirts unfavourable to *Simulium* breeding. Grunebald (1978) asserts that most black flies larvae are very sensitive to water pollution and especially to ammonia content.

The water velocity/depth was also found to be almost the same i.e. between 1.1m/sec to 1.5m/sec and between 15.0cm to 30cm respectively. In addition, a common phenomenon was observed in the distribution of the substrates across the five sites and also the river beddings. The highest numbers of *Simulium* immature stages were caught in the water velocity of between 1.3-1.5m/sec, followed by 1.1-1.5m/sec. This is at variance with the study conducted by Egwumah (1989) in the same river who recorded 1.2-1.3m/sec for *S. hargreavesi* and 1.4-1.5m/sec for *S. damnosum*. As noted by Grunewald (1981), that although water velocity could not be typically considered to be distinctiveness for the diverse cytospecies, it seems to set lesser and higher restrictions from the complex as a whole at 0.4 – 2.4 m/sec and as noted by Onyenwe *et al.* (2007) the data range of 0.55 – 1.24 m/sec from river Eshi are within these limits. The water temperature in this study varied between 16-24 °C increasing from November and decreasing in January. This is almost at the same range with Onyenwe *et al.* (2007) who reported for river Eshi water temperature ranging between 18-30 °C, and also 18.3-31.8 °C as reported by Ocran *et. al.* (1982) in the old OCP area, tallying with the pattern of variation corresponding with the annual cycle of water temperature reported by Ocran *et al.* (1982) who described the coolest temperature in Dec-Feb, and warmest the start of the rains. Although the temperature in Jos starts getting cooler from November to early February and becomes hot again during the peak of the dry season beginning from the end of February to early May. The temperature reported here could probably be one of the lowest temperature at which *S. damnosum* complex were collected as compared to the lowest temperature at which *S. damnosum* complex members have been found breeding is 16.8°C for Sanje in East Africa (Grunewald 1981). But a little lower than reported by Quillévéré *et al.* (1977) who found *S. sirbanum* breeding at temperatures 25-33 °C in Côte d'Ivoire which could probably suggests why we were unable to collect *S. sirbanum*.

CONCLUSION

The study concludes that there was a decline in the number of black flies species larvae (Figure 7), which could be attributed to various factors that includes as earlier noted, water conductivity, temperature and pH of water, climate change, anthropological activities,

competition for space, previous intervention measures and probably evolutionary dimensions. Overall, A sympatric distribution is observed among the species with a gregarious pattern of living and a guild could therefore be inferred.

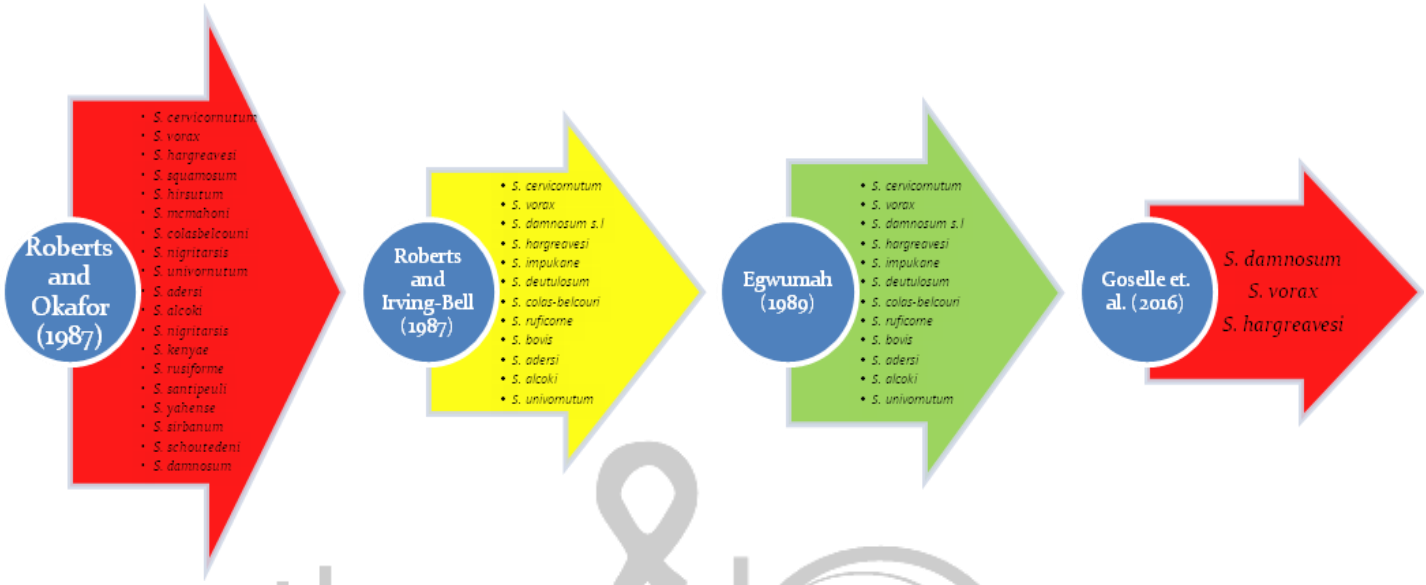


Figure 7: Schematic representation of the decline in black flies larvae in Assop Falls over a three decade period

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 juveniles. BY analyzed the statistical analysis. WAP and HBM aided in the laboratory differentiation of juveniles and writing of manuscript. All authors wrote, read, and approved the manuscript.

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