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Distribution and Abundance of Immature Simulium Species in Oban Streams Cross River State, Nigeria.

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Abstract: *A survey of the distribution and abundance of immature Simulium species was carried out in four streams of Oban, Akamkpa Local Government Area of Cross River State, Nigeria. Larvae and pupae collected were identified as Simulium medusaeforme, S. damnosum, S. alcocki and S. vorax. Of the one thousand one hundred (1,100) immature Simulium species collected between November 2014 and October 2015, 780 (70.91%) and 320 (29.09%) were Simulium larvae and pupae respectively. The larvae of S. medusaeforme (21.41%), S. damnosum (55.51%), S. alcocki (12.31%) and S. vorax (10.09%) were identified. From the 320 pupae, S. medusaeforme (26.8%), S. damnosum (15.63%). S. vorax (35.31%) and S. alcocki (22.19%) were identified. Submerged weeds showed the highest colonization of immature Simulium species compared to submerged rock surfaces, submerged decayed wood and submerged dead leaves. There was significantly higher collection of Simulium larvae and pupae in the dry season in all except Ibe stream. The Shannon biodiversity index was higher in the dry season than the rainy season, with Kwa falls having the highest 1.593 then a decline in Ojuk stream (1.5772), Ibe stream (1.5655) and Mankor stream (1.562). There was statistical significant difference ($p < 0.001$) between the rainy season and dry season in the abundance of immature Simulium species. The seasonal species richness was highest in the dry season in all the streams sampled except Ibe stream.*

Keywords: *Distribution, Immature Simulium, Oban Streams, Nigeria, Biodiversity Index.*

I. Introdction

Black flies otherwise called buffalo gnat are members of the Simuliidae family. They are important arthropods because they vector *Onchocerca volvulus* responsible for human onchocerciasis, a very severely debilitating, blinding disease (Wilson et al., 1994). Black flies are known to exist from the arctic to tropical ecosystems where they have significant economic impact on humans and animal production, and may reduce the fitness of wildlife (Crossky, 1990; Adler et al., 2004). In tropical Africa, onchocerciasis not only incapacitate a large segment of the adult working population, but has prompted whole communities to abandon fertile lands near river valleys in favour of less productive areas in order to be free from the ravage of the disease (Ukoli, 1990).

Many environmental factors including water, temperature, pH, dissolved oxygen concentration, stream width and depth, stream substrates and turbidity, have been known to affect black fly diversity and distribution (McCreadie et al., 2006, Landeiro et al., 2009). Other environmental changes that are associated with enrichment such as alteration of the flow regime and changes in streamside vegetation can also influence the distribution and abundance of black flies (Pachon and Walton, 2011).

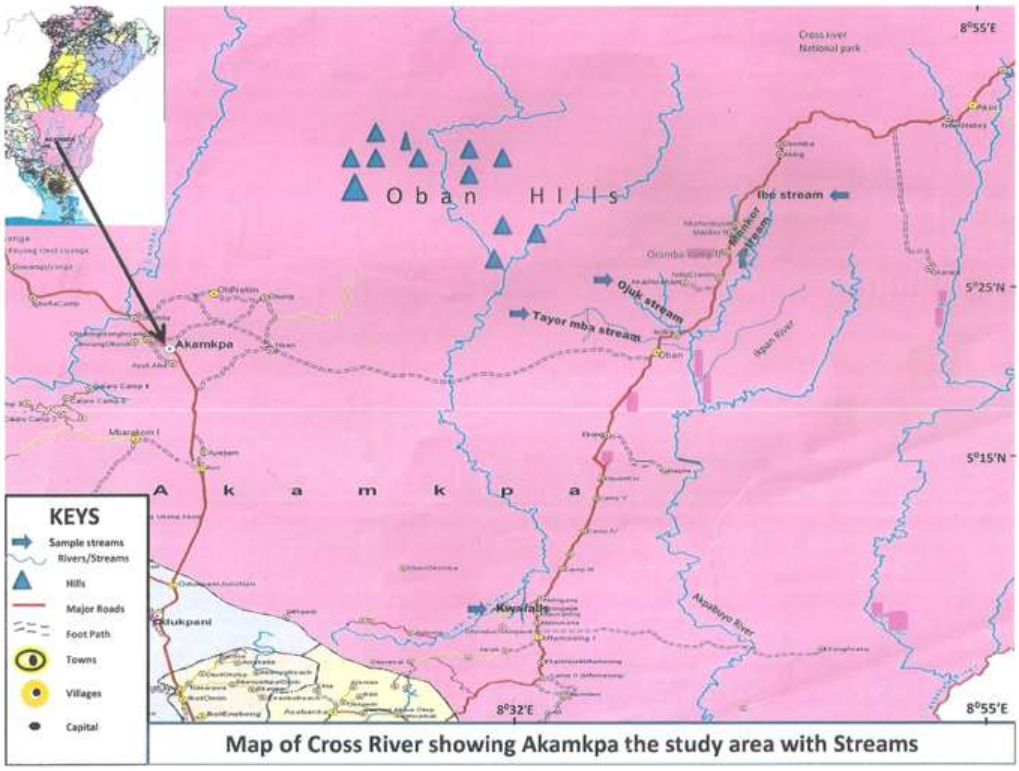
The major vector of Onchocerca volvulus in Nigeria, West Africa and Africa is *Simulium damnosum* complex (Wilson et al., 1994; WHO, 1995, Uzoigwe et al., 2006; Ugwuanyi et al., 2015). Black flies breed in fast flowing points, well oxygenated rivers and streams which are numerous in Nigeria (Crosskey, 1981). Black fly larvae are one of the most important aquatic insect groups, because they play a major role as principal processors of plant materials in running streams (Srisuka et al., 2015). They occupy habitats ranging from temporary stream trickles to large rivers, and are attached to various substrates that are available in streams like fallen leaves, rock surfaces, trailing grasses, tree roots and mud (McCreadie and Adler, 2012; Srisuka et al., 2015). The larvae use tiny hooks at the ends of their abdomens to hold on to the substrate, using silk holdfasts and threads to move or hold their place. They have foldable fans surrounding their mouths which expand to catch debris during feeding (Crosskey, 1962).

The availability of fast-flowing streams due to the topography of the study area, provides adequate favourable breeding sites for *Simulium* species. This study seeks to investigate the distribution and abundance of immature stages of *Simulium* species in Oban streams.

II. Materials and methods

Study area

The study site was Oban streams in Akamkpa, Cross River State, Nigeria. Oban is famous for its Oban Hills, from where the four surveyed streams arose and flow south-westerly into Akpabuyo river (Fig. 1). Oban is located between latitude 5^{o} 15' and 5^{o} 25' N and longitude 8^{o} 32' and 8^{o} 55' E of the Greenwich meridian. The topography of Oban is that of igneous



Map of Cross River showing Akamkpa the study area with Streams

Metamorphic rocks which have given rise to the Oban Hills. The natural vegetation is dense tropical rainforest which is transitional from evergreen to mixed deciduous forest. The dominant occupation of the people is farming of plantain (Musa sapientum) and fishing.

Sample Collection

The sampling method adopted in this study was that of Srisuka et al., (2015). Larvae and pupae were collected from submerged rock surfaces, submerged dead wood, dead decayed leaves and submerged weeds. The larvae from different streams were preserved in separate plastic vials (7.5 cm long and 2.1 cm wide), containing 70 percent ethyl alcohol before transportation to Biological Science Laboratory of Cross River University of Technology, Calabar, for entomological studies. Larvae were identified using keys provided by Crosskey (1962). Pupae were also maintained individually in similar vials under similar conditions before transportation to the laboratory for entomological studies and were identified after De-Mellon (1953).

Species Identification

Keys provided by Freeman and De-Mellon (1953) and Crosskey ((1969) were used for the identification of morphological characteristics of pupae and larvae respectively.

Data analysis

The determination of diversity between streams and seasons were calculated using Shannon Biodiversity index (H), Species richness (S), and Shannon equitability (J) or evenness. The Shannon index is a diversity index, which takes into account the number of individuals as well as that of taxa. Species richness was expressed as the number of observed species. Equitability (J) was calculated based on Shannon diversity divided by the logarithm of the number of taxa. This measured the evenness with which individuals were distributed among the taxa present. The chi-square was also used to determine the distribution and abundance of *simulium* larvae and pupae from Oban streams.

III. Results

Out of one thousand one hundred (1,100) immature *Simulium* species collected between November 2014 and October 2015, from four streams in Oban, 780 (70.91%) and 320 (29.09%) were larvae and pupae respectively (Table 1). *Simulium* larvae were more abundant in November 129 (16.54%), while the least was recorded in June 31 (3.97%). The highest number of *Simulium* larvae 390 (50.00%) was obtained from Kwa falls, followed by Ibe stream 146 (18.72%) then Mankor stream 130 (16.67%) and least in Ojuk stream 114 (14.62%). Kwa falls recorded the highest Shannon diversity index (H) of 1.3984 and Shannon equitability (J) of 1.0087. Decreased in Shannon diversity index was observed in Ibe, Mankor and Ojuk streams and recorded the values 0.7054, 0.6958 and 0.5840 respectively. Shannon equitability (J) decreased similarly in Ibe 0.5088, Mankor 0.5019 and Ojuk 0.4213. There was no larva collected in the months of July, August and September due to high tide (table 1). However, the highest number of pupae 52 (16.25%) was collected in April, and the least 24 (7.50%) in October. Also, Kwa falls had the highest pupae abundance of 134 (41.88%) and decreasing to 75 (23.44%) in Mankor, 63 (19.69%) in Ibe and 48 (15.00%) in Ojuk stream. It was observed that Kwa falls also recorded the highest Shannon diversity index (H) of 1.2536 and Shannon equitability (J) of 0.9043 (Table 1).

Table 2 shows seasonal abundance and identified *Simulium* species from Oban streams. Of the 780 *Simulium* larvae collected, 167 (21.41%) were identified as *Simulium medusaeforme*, 433 (55.51%) as *S. damnosum*, 84 (10.77%) as *S. vorax* and 96 (12.31%) as *S. alcocki*. The 320 pupae collected from sampling, 86 (26.88%) were *S. medusaeforme*, 50 (15.63%) *S. damnosum*, 113 (35.31%) *S. vorax* and 71 (22.19%) *S. alcocki*. There was statistical significant difference ($p < 0.001$) between rainy season and dry season abundance of *Simulium* larvae and pupae (Table 2). Plate1 and 2 shows the pictorial representation of the identified immature *Simulium* species in the study area. The Shannon diversity index in the dry season (November – March) was highest in Kwa falls, followed by Ojuk, Ibe and the least was at Mankor, which represented 1.593, 1.5772, 1.5655 and 1.5625 respectively. The Shannon index in the rainy season (April-October) was in sharp contrast with the dry season, showing the highest value of 1.3419 at Mankor, followed by 1.3064 at Ojuk, 1.2218 at Kwa falls and 1.2 at Ibe (Fig. 2). The seasonal species richness was highest in the dry season in all the sampled streams for *S.* larvae except in Ibe stream, which had a higher value of 54 in the rainy season and 42 in the dry season for *S.* pupae (Fig. 3).

Table 3 illustrates substrate distribution of *Simulium* larvae and pupae in Oban streams. Submerged weeds had the highest colonization 218 (27.95%) of *Simulium* larvae and 150 (46.88%) of *S.* pupae. Submerged rock surface showed the second preferred substrate of habitation as depicted by 198 (25.38%) abundance of *S.* larvae and 80 (25.00%) of *S.* pupae. The third preferred substrate for *S.* larvae was submerged dissolved wood with 190 (24.36%) abundance, while decayed dead leaves with 50 (15.63%) abundance was the preferred substrate for *S.* pupae. The least preferred substrates for *S.* larvae and *S.* pupae were decayed dead leaves and submerged dead wood which shows 174 (22.31) and 40 (12.50%) respectively. The Shannon diversity index (H) of 0.7004 and 0.9547 were the highest in submerged weeds for *S.* larvae and *S.* pupae respectively. In the same pattern, the Shannon equitability (J) of 0.5052 and 0.6887 were also highest in the same substrate for *S.* larvae and *S.* pupae respectively.

Table 1. Monthly sampling of immature Simulium species in Oban streams

	Larvae					Pupae				
	Streams					Streams				
Month	Ibe	Mankor	Ojuk	Kwa falls	Total	Ibe	Mankor	Ojuk	Kwa fals	Total
November	25	22	18	64	129 (16.54)	5	6	5	23	39 (12.19)
December	10	12	14	60	96 (12.32)	9	8	4	12	33 (10.31)
January	18	14	15	54	101 (12.31)	4	8	5	19	36 (11..20)
February	16	18	12	47	93 (11.92)	9	13	3	10	35 (10.94)
March	14	9	8	38	69 (8.85)	7	5	5	15	32 (10.00)
April	31	20	23	40	114 (14.62)	13	10	1	18	52 (16.25)
May	7	15	6	21	49 (6.28))	6	8	6	20	40 (12.50)
June	8	10	3	10	31 (3.93)	5	9	6	9	29 (9.06)
July	0	0	0	0	0 (0.00)	0	0	0	0	0 (0.00)
August	0	0	0	0	0 (0.00)	0	0	0	0	0 (0.00)
September	0	0	0	0	0 (0.00)	0	0	0	0	0 (0.00)
October	17	0	15	56	98 (12.56)	5	8	3	8	24 (7.50)
Total individuals	146 (18.72)	130 (16.67)	114 (14.62)	390	780	63	75	48	134	320
Shannon diversity index (H)	0.7054	0.6958	0.584	1.3984		0.6765	0.8274	0.6028	1.2536	
Shannon Equitability (J)	0.5088	0.5019	0.4213	1.0087		0.4879	0.5968	0.4348	0.9043	

Table 2. Seasonal abundance of *Simulium* larvae and pupae in Oban streams

	Larval species										Pupal species									
	No of larvae collected		*S. medusae forme*		*S. damnosum*		*S. vorax*		*S. alcocki*		No of larvae collected		*S. medusae-forme*		*S. damnosum*		*S. vorax*		S. alcocki	
STREAM	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS	RS	DS
IBE	98	110	11	25	63	62	4	14	20	9	52	42	16	10	6	6	20	14	10	12
MANKOR	49	113	10	32	32	45	4	18	3	18	28	41	5	11	7	7	8	14	8	9
OJUK	31	119	2	29	21	67	3	13	5	10	24	44	6	8	5	9	6	17	7	10
KWA FALLS	114	146	15	43	86	57	5	23	8	23	38	50	10	20	6	4	18	16	4	11
TOTAL	292	448	38	129	202	231	16	68	36	60	142	178	37	49	24	26	52	61	29	42

Key: RD = Rainy Season, DS = Dry Season

Table 3. Substrate abundance of *Simulium* species in Oban streams

SPECIES	LARVAE					PUPAE				
	SRS	SDW	DDL	SW	Total	SRS	SDW	DDL	SW	Total
S. m edusaeforme	36	37	42	52	167 (21.41)	29	8	14	35	86 (26.88)
S. vorax	18	17	22	27	84 (10.77)	8	7	9	28	52 (16.25)
S. damnosum	119	117	90	107	433 (55.51)	38	15	20	72	145 (45.31)
S. alcoki	25	19	20	32	96 (12.31)	5	10	7	15	37 (11.56)
Total individuals	198	190	174	218	780 (70.91)	80	40	50	150	320 (29.09)
Shannon diversity (H)	0.6265	0.6008	0.6031	0.7004		0.6276	0.4283	0.4948	0.9547	
Shannon equitability (J)	0.4519	0.4334	0.4350	0.5052		0.4527	0.3089	0.3569	0.6887	

Key: SRS = Submerged rock surface; SDW = Submerged dead wood; DDL = Dead decayed leaves; SW = Submerged weed.

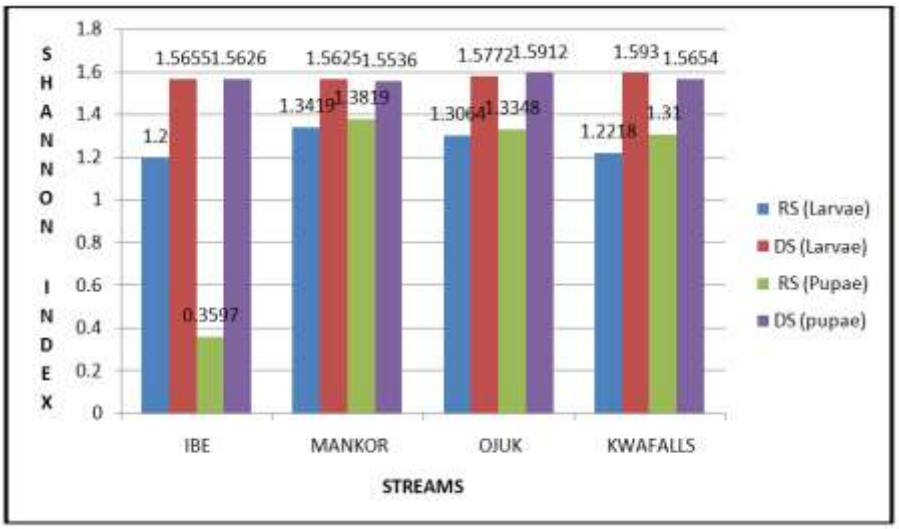
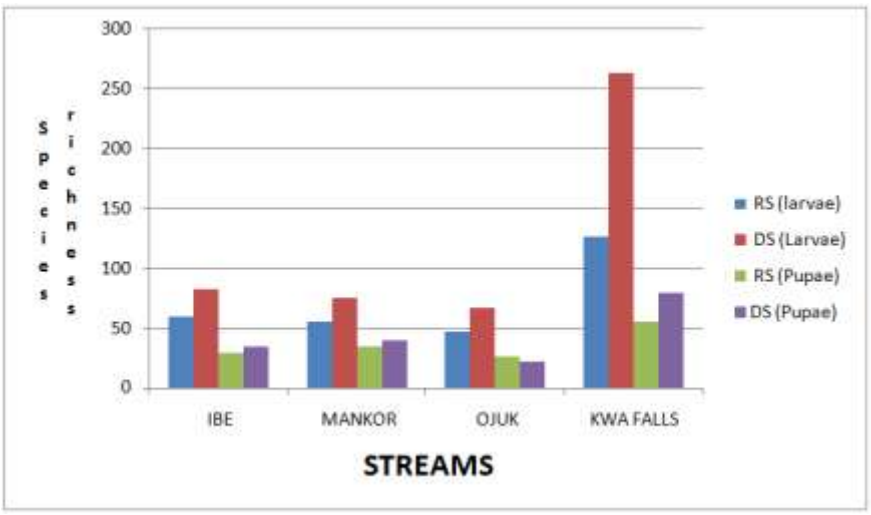
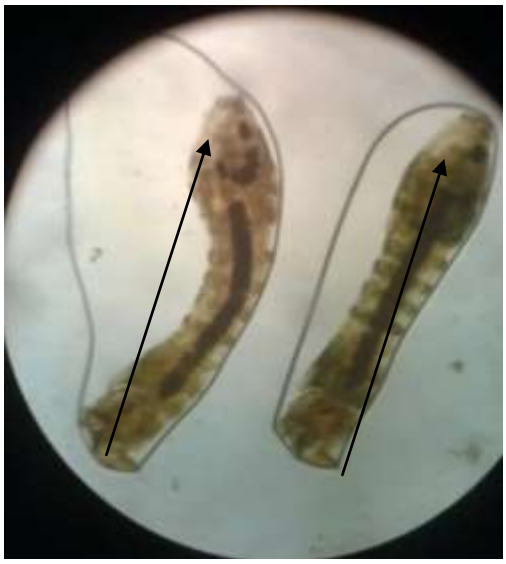


Fig.2. Shannon diversity index (Shannon index, H) in four streams of Oban





***S. vorax* larva X40 *S. damnosum* larva X40 With axe-like posterior**

A pair of *S. meduseforme* X40

Simulium pupa X40

Simulium alcocki X40

Plate 1: Pictorial representation of *simulium* larvae and pupa identified from the study area.

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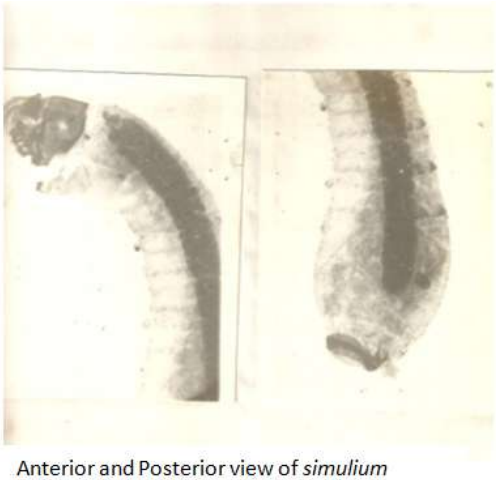

Anterior and Posterior view of *simulium damnosium* larvae X 400

Anterior and Posterior view of *simulium vorax* X 400

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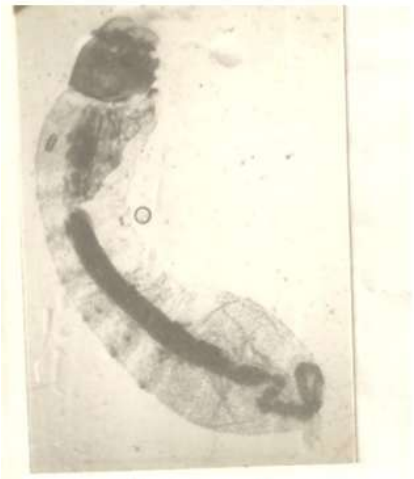

Identified larvae of *simulium medusaeforme* X 400

Identified *simulium alcocki* larvae X 400

Plate 2: Pictorial representation of Anterior and Posterior *simulium* larvae identified from the study area. X 400

IV. Discussion

This study revealed the presence of black flies in Oban streams. The presence of *Simulium* larvae and pupae in the surveyed streams provide evidence of the streams as breeding sites for blackflies. This finding corroborates earlier reports (Braide e al., 1980; Opara et al., 2005; Atting et al., 2005). The distribution of adult female black flies in this area had earlier been reported (Iboh and Arong, 2015). Monthly distribution and abundance of *Simulium* larvae and pupae showed that they were most abundant in April and May during the early rainy season. This finding is in consonance with previous reports (Akpan et al., 2012; Uzoigwe et al., 2013). The changes in water velocity occasioned by the early rains, availability of rapids by igneous rocks underlying the stream beds, provided fast flowing points as breeding grounds for *Simulium* species. This observation is similar to that reported by Akpan et al., (2012) and Uzoigwe et al., (2013). Kwa falls recorded the highest immature *simulium* species (larvae and pupae) compared to Ibe, Mankor and Ojuk streams. This could be attributed to canopy cover, riparian vegetation and water velocity which provided important ecological factors influencing species assemblage (Srisuka et al., 2015). Our inability to record larvae and pupae in the months of July, August and September was not unconnected with inaccessibility of the streams due to excessive flooding. Four species of black flies were identified from the immature *Simulium* species collected. These were *S. medusaeforme, S. damnosum, S. vorax and S. alcocki. S. medusaeforme and S. damnosum* have six dorsal spines on their bodies. However, *S. medusaeforme* was distinguished from *S. damnosum* by the possession of two arms of cephalic fans on the head, unlike one arm of cephalic fan on *S. damnosum* head (Freeman and De-Melton, 1953; Crosskey, 1962). Both *S. vorax* and *S alcocki* are plain without spines on their dorsal surfaces and possess a single cephalic fan. But *S. vorax* was distinguished by its axe-head-like posterior end and a pale colour, unlike *S. alcocki* which was smaller and more pigmented than S. vorax (Freeman and De-Melton, 1953; Crosskey, 1962). Plate 1 shows the pictorial representation of these immature *Simulium* forms from surveyed

streams in Oban community. Prospecting for *Simulium* larvae and pupae in Oban streams is painstaking and captivating, sometimes taking about three hours before discovering their breeding sites. Their sight was interesting whenever any suspected breeding site proved positive for larvae and pupae presence. Most of these (780) larvae and (320) pupae were collected on submerged weeds and submerged rock surfaces at depths of about 60mm below the water surface in fast flowing points. This finding is in line with some earlier published works (Iboh and Braide, 1987; Ibe et al., 2007). The most preferred substrate for colonization was submerged weeds, followed by submerged rock surfaces. The least preferred substrates were decayed dead leaves (DDL) for larvae and submerged dead wood (SDW) for pupae. Although four species of the immature forms were identified, *S. damnosum* was the most dominant species due to the shady canopy along the stream as earlier observed by Srisuka etal.,(2015).

For stream diversity of black flies, the Shannon index and Shannon equitability for larvae and pupae were highest in Kwa falls. It was observed that species richness was highest in Kwa falls than in Ibe, Mankor and Ojuk streams. However, seasonal diversity of black flies revealed that Shannon index and species richness for larvae and pupae were higher in the dry season than the rainy season in all sampled streams. The higher diversity of Shannon index and species richness in the dry season is due to unstable fast running streams and the sloppy topography of Oban, which aid distribution of species diversity after the rainy season. This report is in conformity with the findings of Srisuka et al., (2015), who reported that Diversity is lower in the rainy season than in the cold one, when the flow of water in most streams stable.

In conclusion, this study provided information on the distribution and abundance of *Simuliid* species in Oban streams. Species diversity, richness and seasonal abundance were influenced by ecological factors like natural substrates, water velocity, riparian vegetation and canopy along the streams. However, species richness was observed to be higher in the dry season than rainy season except for Ojuk in pupae.

Competing interest. There was no competing interest in this research work.

Authors' contribution.

CII initiated the concept of this research work. CII and GAA, were involved in sample collection and writing of the manuscript. Approval of the final draft was the responsibility of all the authors.

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