



Morphotaxonomic Studies and Abundance of *Simulium damnosum* Theobald (Diptera: Simuliidae) Complex in Two Local Government Areas of Ogun State

Oyinloye, N.A.,^{1*} Sam-Wobo, S.O.,¹Oke, O.A.,¹Pitan, O.R.,²Surakat, O.A.,³Adekunle, N.O.⁴ and Awoyale, K.A.¹

¹ Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria.

²Department of Crop Protection, Federal University of Agriculture, Abeokuta, Nigeria.

³Faculty of Basic and Applied Sciences, Department of Zoology, Osun State University, Osogbo, Nigeria.

⁴Department of Plant and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria

Abstract

The sibling species composition of *Simulium damnosum* sensulato and abundance of these sibling species in two communities (Opeji and Imala-Odo) of Ogun State south-western Nigeria was carried out in the wet months (May to October). Morphological identification of black flies using standard protocol was done from 07:00hr-18:00hr twice every two weeks. Climatic data like temperature, rainfall and relative humidity were documented for each month. A total of 1543 flies were dissected, 1228(79.6%) in Opeji and 315(20.4%) in Imala-Odo with a statistical significance ($p<0.05$). The result revealed an allopatric existence of forest dwelling flies. All the flies identified had dark fore coxa but there was variation in the colours of their wing tuft where wing tuft grade 05 was predominant and no fly had wing tuft grade 01 and there was significant difference ($p<0.05$) in the wing tuft colours observed among the flies. In Opeji, 477(38.8%) flies were parous while 155(49.2%) parous flies were recorded in Imala-Odo. However, no black fly was infected with the larva of *Onchocerca volvulus*. Relative diurnal distribution of flies caught revealed a bimodal peak between 10:00-11:00hr and 15:00-16:00hr in both communities. Monthly data on abundance also revealed a bimodal peak in the study sites in June and September with a significant difference ($p<0.05$) in the abundance across the months. Highest biting rate was recorded in June (Opeji=4492.5; Imala-Odo=780) while August recorded the lowest monthly biting rates (Opeji=284; Imala-Odo=62). The relationship between flies abundance and climatic data showed no significant relationship in both communities ($p>0.05$). This study revealed that there is high abundance of forest black flies during wet season which have epidemiological implication on humans living in these areas and efforts should be made towards constant monitoring of the siblings species.

Key words: *Simulium damnosum*.l., morphotaxonomy, abundance, biting rates, parity

*Correspondence email:
naomiyoynloye@gmail.com
ORCID: 0000-0002-4347-4324

INTRODUCTION

Black flies have been incriminated as the vectors of river blindness (onchocerciasis)

in different parts of the world (Brockhouse *et al.*, 2011; WHO, 2017; WHO, 2022). Black flies breed in fast-flowing streams and rivers with the velocity of about 50 centimetres per second (Leveque, 1989). The blood feeding activity of adult females transmits several pathogens to humans, most notably *Onchocerca volvulus*, the causative agent of

river blindness (Adler, 2005). An estimated 198 million people live in areas where they are at risk for onchocerciasis (WHO, 2017). The presence and abundance of *Simulium damnosum* has been reported to be directly related to the prevalence of human onchocerciasis (WHO, 2017).

The West African Blackflies habitat is characterised by vegetative zones (either as savanna or forest), the topography of the area, the rivers size that support fly breeding and other factors which influence the distribution dynamics of transmission (Boakye *et al.*, 1998). These species *S. sirbanum*, *S. damnosum* *ensustricto* and *S. dieguerense* are known as savannah flies which transmit savannah strain of *O. volvulus*. *S. sanctipauli*, *S. soubrense*, *S. squamosum*, *S. yahense*, *S. leonense* and *S. konkorensis* belong to the forest group and transmit the forest strain of *O. volvulus* of which the pathogenicity is more of skin disease with less blinding (WHO, 1995; Ibeh *et al.*, 2006; Adeleke *et al.*, 2010a). Therefore, a reliable identification of biting females of *S. damnosum* complex that transmit the forest and savannah strains of *O. volvulus* has been considered very essential in the study of transmission and epidemiology of human onchocerciasis (Ibeh *et al.*, 2008). WHO has verified four countries in the Americas as free of onchocerciasis after successfully implemented elimination activities (Colombia in 2013, Ecuador in 2014, Mexico in 2015 and Guatemala in 2016), it is therefore necessary for large areas in Africa to map and assess whether transmission is active so as to shift from control to elimination (WHO, 2017; WHO, 2022).

Various members of *S. damnosum* complex have been identified using micromorphological chromosome characters but there are also differences in their biology, anthropophily and vector-parasite interactions which results in differences in overall

contribution of each cytospecies to disease transmission (Mustapha *et al.*, 2004; Surakat *et al.*, 2016). The importance of the different species relates to their capacity for transmission of the disease, and also the ease of control. Where vector control/elimination has been the main tool in the control of onchocerciasis, it has been important to understand the detailed biology of each species, to establish the best method of control (Adrian and Boakye, 2011). A detailed morphological study of the adults of the six common sibling species of the *S. damnosum* complex occurring in West Africa has been in progress since 1976. Its purpose was to investigate external characters that could be relatively easily observed and used with a high degree of reliability to separate these species (Garms, 1982).

Morphological identification of the siblings of *S. damnosum* complex was reviewed by Garms *et al.*, (1982) who developed methods for morphological identification of adult female black flies based on earlier works of (Kurtak *et al.*, 1981). The use of morphological characters could allow the identification of *S. yahense* with approximately 99% specificity while savanna cytospecies (*S. damnosum* *s.* and *S. sirbanum*) could be easily separated from *S. sanctipauli* subcomplex (*S. sanctipauli* and *S. soubrense*, *S. leonense* and *S. konkorensis*) and *S. squamosum* subcomplex (*S. squamosum* and *S. yahense*) using the thorax-antenna length ratio (morphometrics), colour of wing tuft, fore coxa and abdominal tergite (Garms *et al.*, 1982; WHO, 1995; Mafuyai *et al.*, 1996; Mank *et al.*, 2004). However, since this method allows only one species of the complex to be distinguished and gross grouping of other species into savanna or forest flies, accurate identification of adult flies on field still remained unresolved and could lead to various degrees of misidentifications (Mafuyai *et al.*, 1997).

Despite these limitations, morphotaxonomy remains the rapid method of grossly distinguishing members of *S. damnosums*. 1. in the field (Ibeh *et al.*, 2008). Therefore, this study aims to determine the biting habits and transmission potentials of *S. damnosum* complex in Ogun State using dissection method.

MATERIALS AND METHODS

Study areas

The study was conducted in two Local Government Area Opeji in Odeda Local Government Area and Imala-Odo in Abeokuta North) of the eight Local Government Areas of Ogun State currently receiving annual ivermectin treatment through Community Directed Distributors (CDDs). Opeji community is located on latitude 003.37194 and longitude 07.32048, it

is inhabited mainly by Yoruba, Hohori and Fulani herders groups. Imala-Odo community is located on latitude 003.18154 and longitude 07.32245, mainly occupied by the Ilaje, Agatu and few Fulani herders groups. Members of both communities occupation are mainly fishing and farming. Derived savannah (forest interphase) with patchy forest galleries are the most common vegetation in the two communities. The communities are associated with river courses with the presence of rocky substrata which create rapid flow of water and make them serve as potential breeding sites for *Simuliu mdamnosaurs*.1.

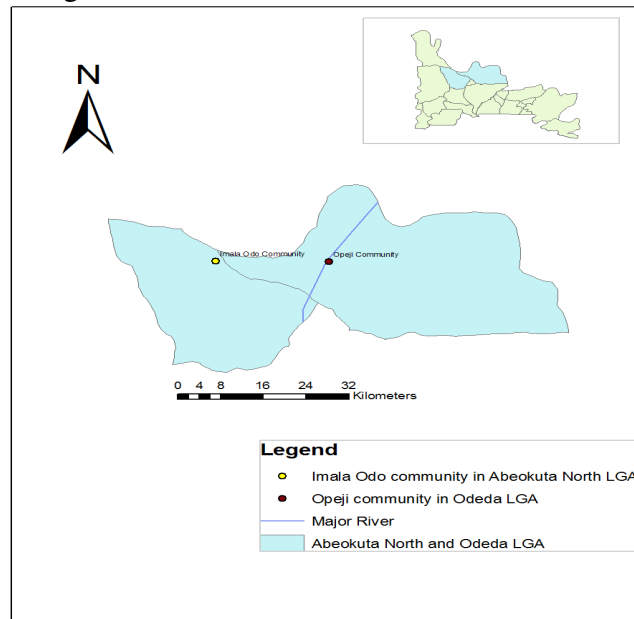


Fig. 1: Map of Ogun State showing study sites

Ethical Approval

Approval for this study was obtained from the Ethics Review Board (ERB) of Ogun State Hospital Management Board, Abeokuta (SHA/RES/VOL2/153). Informed consent was duly signed by community heads and volunteer fly catchers who receive annual dosage of onchocerciasis treatment with ivermectin.

Adult Black fly collection

Blood seeking adult female of *S. damnosums. l.* flies were caught on human baits. Two fly catchers were stationed alternatively for one hour from 07:00 to 18:00 hour between May and October near the bank of the river and farmlands close to the bank of Opeji River in Opeji community and Oyan River in Imala-Odo community twice every two weeks in each study location. The fly catchers sat at the catching points and exposed the lower part of their legs. Flies coming to perch on the exposed parts were caught individually before it procures a blood meal by inverting a small plastic tube over it and the cap of the tube immediately replaced. The tubes were pooled according to the hour of catch and recorded accordingly.

Black fly identification

All hourly-caught black flies were individually anaesthetized using chloroform. The flies were identified with the aid of a dissecting microscope (Wild Heerbrugg M1B; Binoculars x 8/21) and classified as forest dwelling flies considering features such as colour of the first four posterior antennae segments, ninth abdominal tergite setae, scutella setae and colour of fore coxa of each of the fly was observed and scored as pale, dark or intermediate as described by Garms *et al.*, (1982). The wing tufts (stem vein setae) were also observed and scored on a scale of 01-05 and 00 according to Mafuyai *et al.*,

(1996), modified by Garms *et al.*, (1982) and adapted by WHO (1995).

Physiological age composition of the flies

Each identified flies caught were placed on a microscope slide with a drop of normal saline and was cut open on the third segment of the abdomen under a dissecting microscope using dissecting pins. The ovaries of the dissected flies were stretched and classified as parous or nulliparous after observing other characters like absence or presence of fat bodies and the colour of malphigian tubules as described by (Oluwole *et al.*, 2009; Adeleke *et al.*, 2010a). For nulliparous flies, the ovaries had tightly coiled tracheal systems and there was absence of follicular relics. Presence of fat bodies and cloudy malphigian tubules indicated that the flies had not taken blood meal and may not have parasite larvae.

Monthly Biting Rate: The monthly biting rate (MBR) of flies caught using normalized catching method was estimated in accordance with (WHO, 1995).

$$\text{MBR} = \frac{\text{Number of black flies} \times \text{Number of days in the month}}{\text{Number of catching days in a month}} \times 100$$

Parous Rate: parous rate is estimated as the percentage of parous females in a sample of dissected female flies.

Climatic data of study area

Monthly average rainfall, monthly average relative humidity and monthly average temperature of Abeokuta were collected to determine their influence on abundance of *S. damnosums. l.* the data were collected every month from the Hydrology Department of Ogun-Oshun River Basin Development Authority Headquarters in Abeokuta, Ogun State.

Data analysis

A t-test was used to compare the abundance of forest flies in both catching sites and the relationship between monthly abundance and climatic data was analysed using Pearson's correlation analysis. The cut-off was taken as 5% and all data were analysed using Statistical Package for Social Sciences (SPSS) version 20.0.

RESULTS

A total of 1543 host seeking black flies from normalized catch were collected

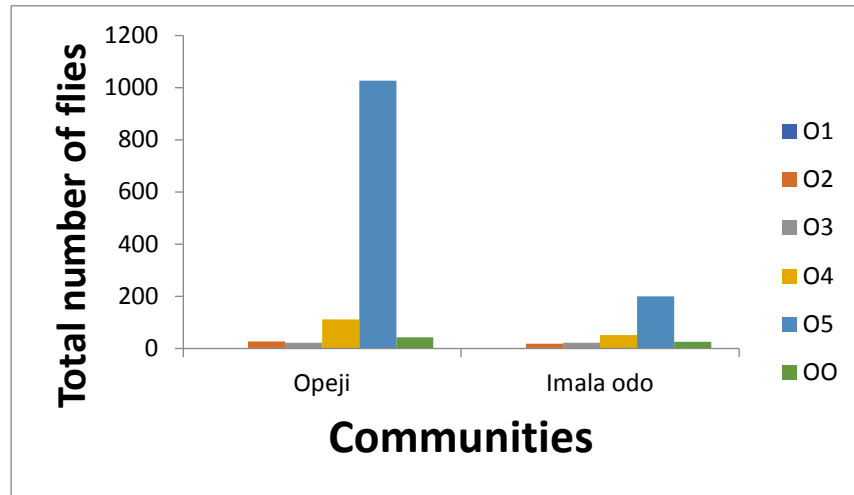
during the study period, of which 1228 (79.6%) were caught in Opeji while 315 (20.4%) were caught in Imala-Odo. There was a statistically significant difference ($p < 0.05$) in the density of flies caught in both communities as shown in table 1. None of the parous flies were found to be infected with larva of *O. volvulus* while one (0.08%) of the nulliparous flies was found to be infected with microscopic nematode mermithid as shown in fig. 2



Fig. 2: A nulliparous black fly found with adult mermithid under the microscope

The result of morphotaxonomy revealed that all flies caught in both communities belong to the forest group with black fore coxa, pale colour of first two to three posterior antennae segments, dark scutellum setae and a mixture of dark and pale ninth abdominal tergite setae. However, different variations in the wing tuft colours were observed among flies in both communities. Flies with grade 05 wing tufts

had the highest number of occurrences in both locations followed by grade 04 and flies with grade 03 wing tufts recorded the least in Opeji community as against grade 02 wing tuft in Imala-Odo (Fig. 3). The variation among the wing tuft of the flies was observed to be significantly different with $p < 0.05$ in and between both study sites.



O1: All wing tufts pale; O2: More pale wing tufts with few dark ones; O3: Mixed wing tufts; O4: More dark wing tuft with few pale ones; O5: All wing tufts dark; O0: No visible wing tuft.

Fig. 3: Variations in the colour of wing tufts of black flies found in Ogun State

Table 1: Entomological features of *Simulium damnosums*. 1. caught in Ogun State

Entomological parameters	Community	
	Opeji (N=1228)	Imala-Odo (N=315)
Parity/physiological age		
Number (%) of Nulliparous flies	751(61.2)	160 (50.8)
Number (%) parous flies	477 (38.8)	155 (49.2)
Total flies (%) Dissected	1228 (100.0)	315(100.0)
Infection status		
Number (%) flies infected with <i>O. volvulus</i>	0 (0.0)	0(0.0)
Number (%) of flies infected with mermithid	1(0.08)	0(0.0)

The highest monthly biting rates (MBR) was recorded in June for both communities (Opeji=4492.5; Imala-Odo=780) while

August recorded the lowest monthly biting rates (Opeji=248; Imala-Odo=62) as shown in table 2.

Table 2: Relative abundance, Monthly Parous Rate and Monthly Biting Rate (MBR) of *Simulium damnosum* s. l. caught in two communities of Ogun State, Nigeria.

Months	Communities	Total flies caught	MPR (%)	MBR	Monthly Average Temp (°C)	Monthly Average rainfall (mm)	Monthly Average Relative Humidity (%)
May	Opeji	126	38.9	976.5	32.12	109.1	81.75
	Imala-Odo	51	33.3	395.3			
June	Opeji	599	33.9	4492.5	38.84	154.7	86.44
	Imala-Odo	104	60.6	780.0			
July	Opeji	241	42.3	1867.8	34.14	82.5	86.66
	Imala-Odo	44	52.3	341.0			
August	Opeji	32	46.9	248.0	34.30	33.00	88.08
	Imala-Odo	8	62.5	62.0			
September	Opeji	190	45.8	1425.0	35.00	176.30	87.70
	Imala-Odo	48	45.8	360.0			
October	Opeji	40	52.5	310.0	33.95	264.7	84.20
	Imala-Odo	60	41.7	465.0			

MBR (Monthly Biting Rate); MPR (Monthly Parous Rate); % (percentage); °C (degree Celsius); mm (Millimeters)

The hourly distribution of flies caught in both communities (Figure 4) showed a bimodal peak with morning peak observed between 10:00-11:00hrs while a afternoon peak between 15:00-16:00hr was observed in Opeji while the afternoon in Imala-Odo was observed between 15:00-16:00 and 16:00-17:00hrs. The monthly data on abundance

(Figure 5) revealed a bimodal peak in the study sites during the study period. The first peak was observed in the month of June (Opeji=599; Imala-Odo=104), the second peak was observed in September while August recorded the least density of flies caught in both sites.

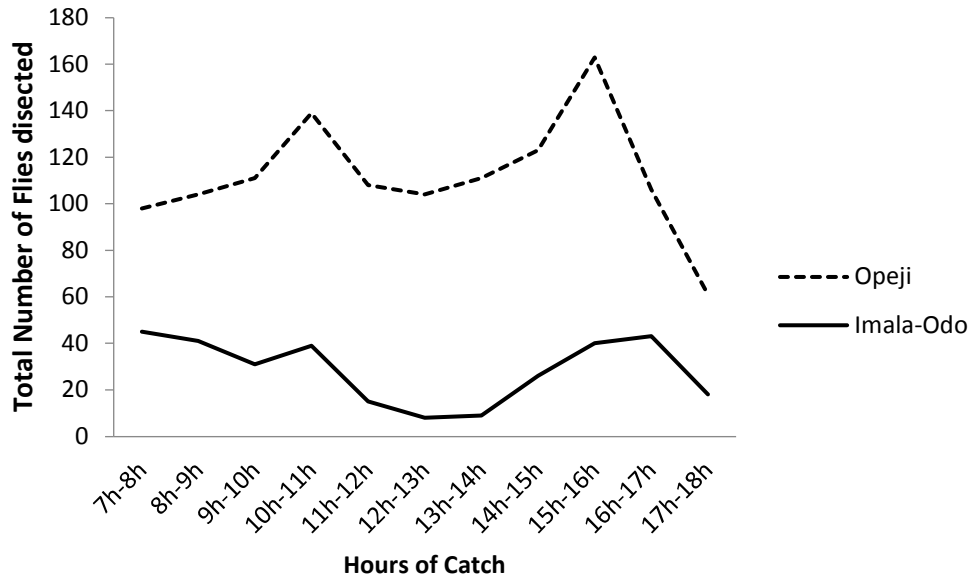


Fig. 4: Hourly distribution of *S. damnosum* l. in Ogun state

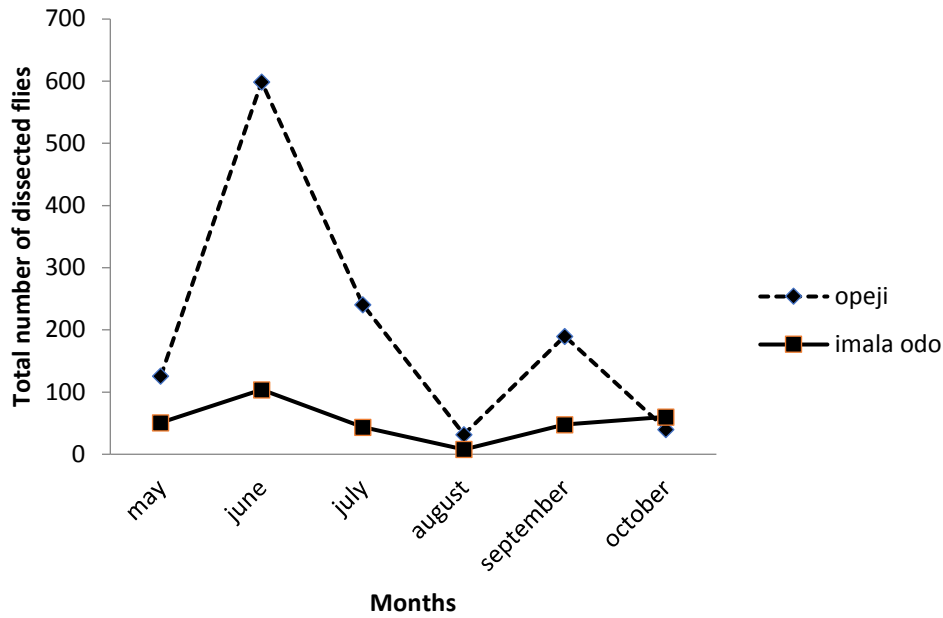


Fig.5: Variations in Monthly density of host seeking black flies in Opeji and Imala-Odo communities, Ogun State

The total hourly biting figures of parous *Simulium damnosum* l. for all the months were pooled together. Two peaks (Figure 6) were observed in Opeji community

between 10.00hr-11.00hr and the highest peak was observed between 15.00hr-16.00hr after which there was a decline in biting activities. A peak was observed in Imala-Odo in the

morning 19 flies between 9.00hr-10.00hr with a decline towards midday and two peaks (19 flies between 15.00hr-16.00hr and 18 flies

between 16.00hr-17.00hr) was observed during the afternoon

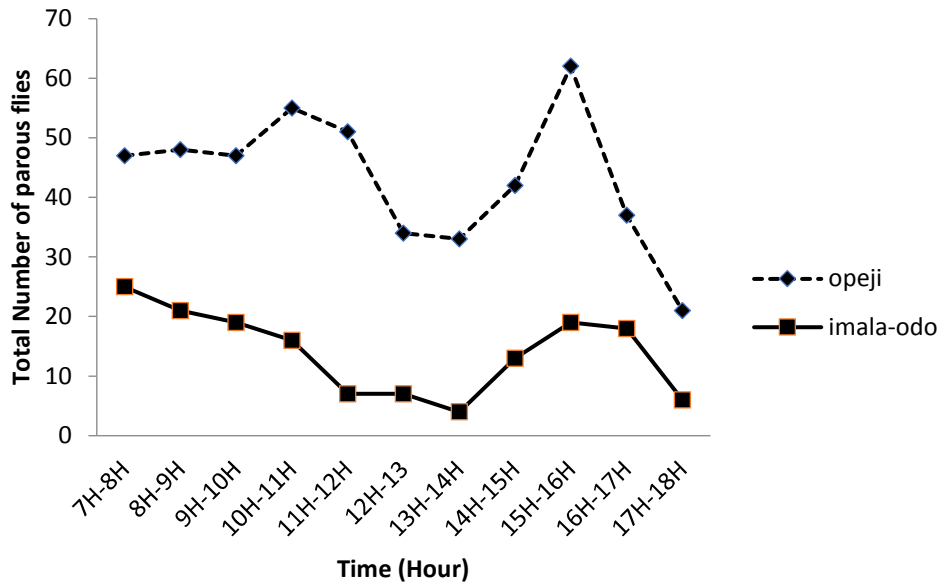


Fig. 6: Hourly distribution of parous *S. damnosum* in Ogun State

Variations in the monthly population of host seeking parous black flies showed that Opeji and Imala-Odo recorded the highest number of hosts seeking parous *S. damnosum*s. 1. in the month of June (203 flies

and 63 flies respectively). The least population of host seeking parous black flies was recorded in the month of August (Opeji; 15 flies and Imala-Odo; 5 flies) as shown in Figure 7

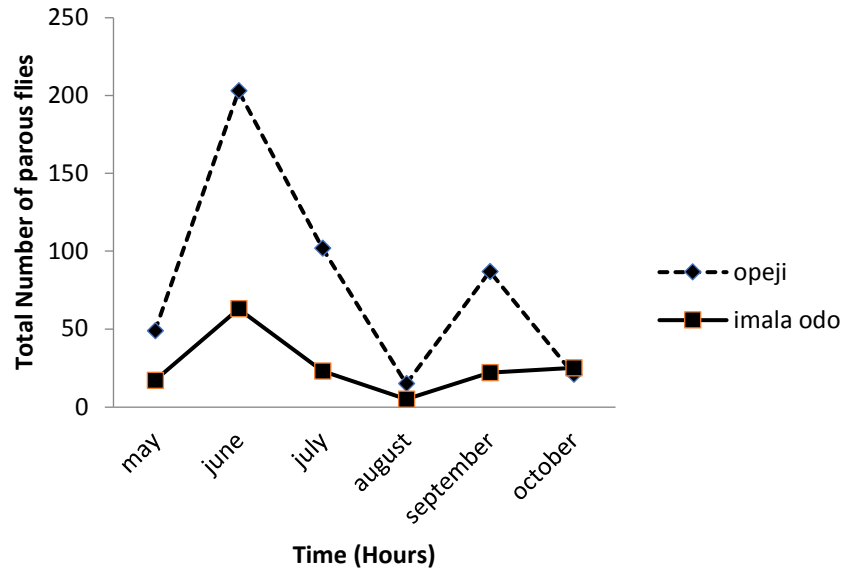
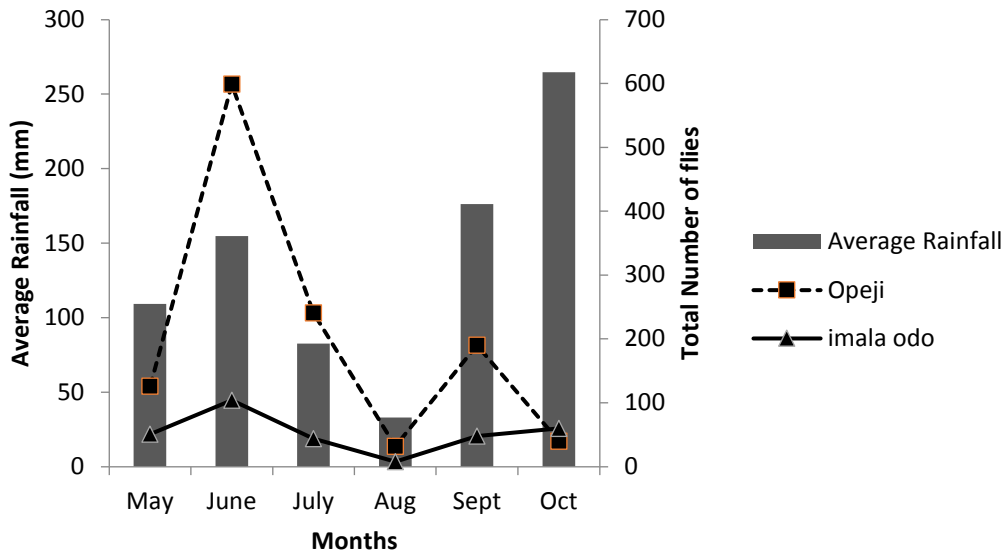


Fig. 7: Variations in Monthly density of host seeking parous black flies in Opeji and Imala-Odo communities, Ogun State

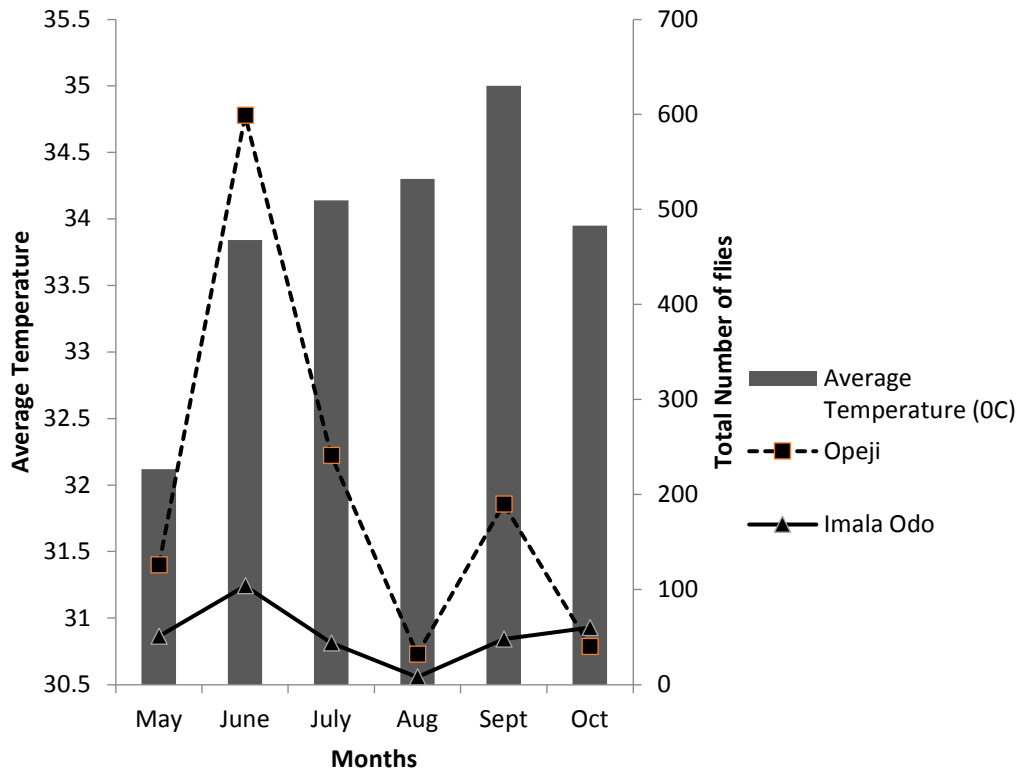
The relationship between monthly climatological data and abundance showed that there was no significant relationship between monthly rainfall and fly population in both communities (in Opeji, $r=0.042$; (Fig. 8) $p>0.05$ and Imala-Odo, $r=0.546$; $p>0.05$). There was no significant relationship between average monthly temperature and fly population in both communities ($p>0.05$) although, positive correlation was observed in

Opeji $r=0.031$ (Figure 9) but a negative correlation was observed in Imala-Odo $r=-0.167$. A negative correlation was also observed between monthly fly population and monthly average relative humidity (Fig. 9) in Imala-Odo ($r=-0.243$) while a positive correlation was observed in Opeji ($r=0.176$) with no significant difference in the relationship ($p>0.05$).



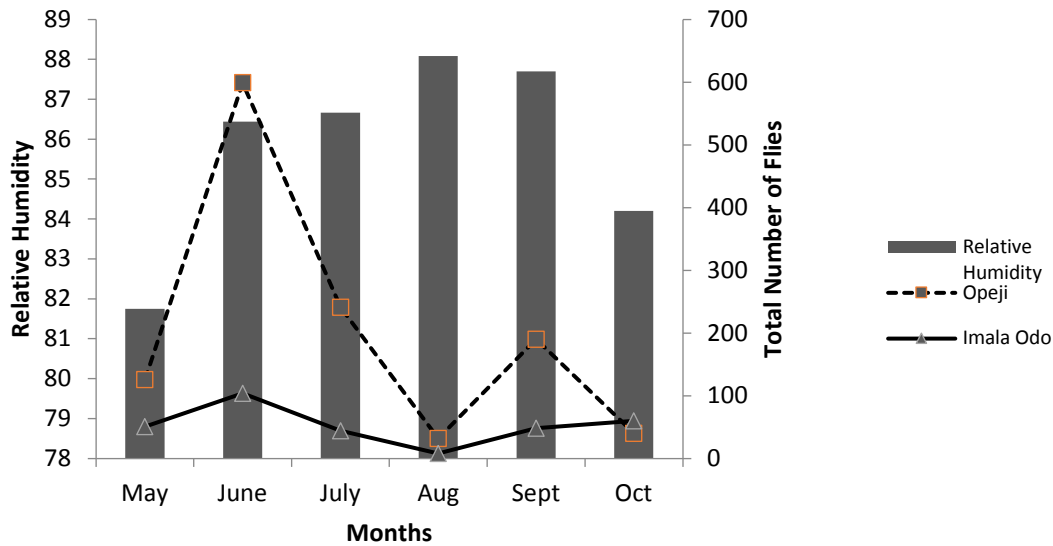
Opeji ($r=0.042$, $p>0.05$); Imala-Odo ($r=0.546$, $p>0.05$)

Fig. 8: Monthly abundance of *S. damnosum* l. with monthly rainfall



Opeji ($r=0.031$, $p>0.05$); Imala-Odo ($r=0.167$, $p>0.05$)

Fig. 9: Monthly population of *S. damnosum* l. with monthly average temperature



Opeji ($r=0.176$, $p>0.05$); Imala-Odo ($r=0.243$, $p>0.05$)

Fig. 10: Population of *S. damnosums*, l. when compared with monthly average relative humidity

DISCUSSIONS

The results of morphotaxonomic studies revealed an allopatric composition of forest dwelling flies in the two study sites. This is expected as Ogun state is located in the forest zone of Nigeria and the study was conducted during the wet season (May – October). This result however agrees with the findings of Adeleke *et al.* (2010a) where only forest flies were reported in the wet months but in contrast with the findings of Oluwole *et al.* (2009) (morphotaxonomy) and Sam-wobo *et al.* (2013) (cytotaxonomy) where they observed a sympatric composition of both forest and savanna dwelling flies in all catching months (wet and dry) in studies conducted in south western Nigeria. Although all flies caught in this study belong to the forest group “07” using standard protocol, variations were observed in the wing tuft composition ranging from 01-05 and 00. This variation is a limitation in using

morphotaxonomy as a classification procedure as it cannot be used to ascertain whether these flies actually differed in species composition or the variation is an outward phenotypic expression of offspring as earlier observed by Adeleke *et al.* (2010a). The absence of black flies with completely dark wing tuft, dark fore coxa and dark colour of antennae segments (forest fly 08) in this study eliminates the possibility of having *S. yahense* in the study areas as these characters are used to unmistakably distinguish this sibling species from other siblings (WHO, 1995). The forest species suspected to be present in the study sites are *S. squamosums*, l. and/or *S. soubrense* because *S. soubrense* (beffa form) was reported by Sam wobo *et al.* (2013) to be dominant forest cytospecies using Heteroduplex assay for identification in a study conducted along Ogun and Yewa river systems. This is in contrast with the findings of (Lamberton *et al.*, 2014) in a study

conducted in Ghana where *S. sanctipauli*, *S. squamosum* and *S. yahense* where the forest cytospecies reported and this could be due to distribution of varying sibling species in different localities and bioclimatic zones.

Relative diurnal biting activity of host seeking *S. damnosum* in both communities showed a bimodal peak in the morning and evening hours as *S. damnosum* are known to bite continuously from dawn to dusk, but not at the same sustained level throughout the day (Adewale *et al.*, 1999). This finding correlates with reports of similar studies in the forest zones (Okolo *et al.*, 2004; Opara *et al.*, 2005; Opara *et al.*, 2008; Chikezie *et al.*, 2015; Akinsanya *et al.*, 2019; Oforka *et al.*, 2020). This variation may be due to sudden changes in weather condition such as temperature, humidity and light intensity (that was often observed during the days of fly collection) which also includes exceptional cool days or sunny days which often affect biting activity of *S. damnosum*. l. (Ubachukwu and Anya, 2001). Although the highest number of hosts seeking *S. damnosum*. l. was caught in June for both study sites, there was significance ($p < 0.05$) in the total number of black flies which may be dependent on the ability of local breeding sites to support black flies production and possibly proximity of the study sites to nearby breeding sites. A bimodal monthly peak was also observed during the study period in the month of June and September.

Variations between monthly biting rates (MBR) within each study sites MBR between both study sites were significant ($p < 0.05$) and this may be due to the rate of local production of breeding sites. These variations can also be attributed to changes in climatic condition of study sites. The highest MBR of 4492 bites/person/month estimated in June for Opeji community is greater than the WHO recommended annual biting rate (ABR) of 1000 bites/person/year in endemic areas

(WHO, 1995) while the highest MBR of 780 bites/person/month was also estimated for Imala-Odo in June.

The relationship between monthly rainfall and relative abundance of host seeking *S. damnosum*. l. caught showed no correlation $p > 0.05$ as against the earlier report of Opara *et al.*, (2005) where there was an increase in both rainfall and abundance during wet season. This deflection may be due to change in climatic condition of the study sites. The sudden decline in the abundance of black flies in August could be due to low rainfall experienced in late July and August as the breeding distribution of *S. damnosum* complex is generally determined by changes in river volume and physical characteristics, especially when seasonal rainfall determines the river volume (Ibeh *et al.*, 2007) while the decline in October could be due to heavy rainfall in late September and October that must have washed away pre-imaginal stages from the colonized breeding sites. Also, the high abundance of black flies caught in June may be due to the stimulus of increased oxygen content of the rivers which cause fly to emerge from pupae (Adewale *et al.*, 1999). Increased oxygen content in breeding sites may be accompanied by increased amounts of nutrients and availability of pre-imaginal development of *S. damnosum*. l. which then results in an increase in the adult black fly population in the rainy season (Opara *et al.*, 2008).

Investigation into the biting pattern of parous host seeking *S. damnosum*. l. in the study sites revealed more biting activity in the mornings with afternoon drop in biting proportions which was later increased in the late afternoon hours. This implies that parous black flies which are able to carry *O. volvulus* larva and they must have completed a gonotrophic cycle are found to be abundant at hours when farming and fishing activities in the study sites is at its peak. This pattern

exposes human population in the study sites to risk of getting infected. The collection of *S. damnosums* l. in mornings would increase the chances of sampling parous flies, and therefore, the likelihood of collecting black flies infected with *O. volvulus* (Adeleke *et al.*, 2010b; Lamberton *et al.*, 2014). The high percentage (61.2%) of nulliparous to parous (38.8%) flies caught in Opeji and infection of one of the nulliparous flies with a microfilaria Mermithid combined with the presence of pre-cambrian rocks and vegetation which caused rapids in the river is an indication of local production of *S. damnosums* l. Mermithids feed on black fly ovaries and so can only be found in nulliparous flies (WHO, 1986) thus, its presence acts as natural agent of control.

CONCLUSION

Forest strains of *Simulium damnosums* l. was identified using morphotaxonomic parameters. Their abundance was recorded and tested against climatological data like temperature, rain fall and humidity and correlations was observed in the study. It is therefore, important to monitor the species composition of *S. damnosums* l. in endemic areas so as to be informed if there has been a change in the composition of various sibling species composition which in turn is useful in monitoring the changes in epidemiological pattern of disease transmission

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