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A survey of six different water bodies in Port Harcourt municipality for larval stages of dipteran vectors.

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Abstract: *Species identification, relative abundance, local distribution, seasonal abundance and similarity index of immature dipteran vectors were investigated from June, 2011 to May, 2012 in six water bodies located in Port Harcourt and Obio/Akpor local government areas of Rivers State. A standard dipping method described by Service, 1971, was used to sample the six aquatic habitats located in two local government areas for dipteran larvae. In summary, 400ml plastic dipper was used to collect water from randomly selected points on the river. Dipping was done in five replicates and the contents sieved in a filter net. The larvae collected were sorted according to their families; a few were selected amongst the families to be reared to adults for further identification and confirmation while others were preserved in 80% ethanol. Collection for dipteran larvae was done bi-monthly from June 2011 to May 2012 between 8am to 12 noon. The larvae and adults were identified using identification keys by Mendes (2011) and already identified and preserved insect types. A total of 1305 larvae and adult stages of Dipteran vectors consisting of 7 species were recorded in all the six sites. Of these, 28.28% were Anopheles gambiae* s.l. *comprising of 369 individuals , 11.03% were Musca domestica (144 individuals) , 39.54% were Culex quinquefasciatus (516 individuals), 3.45%, 3.45% were Stratiomys longicornis (Plate 14) (45 individuals), 1.99% were Ablabesmyia sp(26 individuals), while 9.04% were Sarcophaga haemorrhoidalis (Plate 17) (118 individuals) and 6.67% were Chrysomya bezziana (87 individuals) respectively. The overall abundance of the Dipterans from the various study sites revealed significant differences (P<0.05) among the sites. A posteriori test showed that the abundance of Diptera obtained at Site A was significantly higher than other sites. Jaccard's similarity index revealed similarity between all the sites studied except Site B that served as control. These findings suggest that the pollution associated with urbanization will increase vector densities in Nigerian cities and that urban vector control will become increasingly relevant in few years to come. It is noteworthy however, that poverty, deteriorating infrastructure and overcrowding are some of the factors that contribute to the development of conditions that modify vector breeding sites. As more people move into cities and industrialization proceeds, vector borne diseases will be on the increase in Nigeria.*
Keywords: *Dipteran, vectors, waste, water bodies.*

I. Introduction

The Diptera or "true flies" are one of the largest and most diverse orders of insects, both morphologically and biologically. The order name means "two-winged" and refers to the fact that the hind pair of wings is greatly modified and reduced. The number of described species worldwide is estimated to be 120,000 of more (Stone *et al*., 1965). Although flies with medical or veterinary significance constitute only a small fraction of these numbers, their diversity is impressive, ranging from mosquitoes to wingless ectoparasites, larvae that parasitize various animals and species that help to decompose carrion or feaces (Mendes, 2011). Diptera considered aquatic have aquatic larvae and pupae with terrestrial adults. They inhabit a wide range of aquatic habitats and some taxa are extremely tolerant and occur in extremely polluted water bodies (Xiao-Bo *et al*., 2012)

Ecosystem changes caused by anthropogenic activities have modified the environment in ways that promote the abundance of vectors especially of the order Diptera (Popoola and Otalekor, 2011). Urbanization needs such as housing, agriculture; infrastructural developments mostly unplanned have facilitated the increase in vector population leading to increases in the emergence of vector borne diseases (Nabar *et al.,* 2011). As a result, it is necessary that health care institutions and the academia embark on environmental surveys and research to ascertain the vectors present in these water bodies surrounding urban cities. It is against this background that this work was carried out to determine the Dipteran vectors associated with six water bodies around the Port Harcourt city.

II. Materials And Methods

Study Area

Six streams located in two Local Government Areas (Obio-Akpor and Port Harcourt) were selected for the study. In this study, polluted water bodies were defined as water bodies that had sewage, garbage, oils, and/or other debris at the time of sampling. Sampling for Dipteran vector larvae was done from June 2011 to May 2012. The description of the individual sites labeled A, B, C, D, E and F are as follows:

Site A (Rumuokoro river): is located on latitude 04^0 52'N and longitude 06^0 60'E and is 21.03m elevation above sea level. An abattoir is located besides the stream. Effluents from the abattoir and the general market area flow into the stream. Market shops and residential area also border the stream. A bridge runs across the river at the points of sample collection. Residential houses were situated 23m away from the water body.

Site B (Rumuola river): A police station is 20m from the water body that falls within latitude 04^0 52.3'N and longitude 06^0 52.4'E, this site is 46.63m above sea level. The river is a lotic water body with minimal organic waste. This site served as control.

Site C (Ntanwogba river): is located on latitude 04^0 47.7'N and longitude 07^0 0.2'E has an elevation of 15.54m above sea level. Municipal wastes can be found in the water body. Oil films covered the water body throughout the duration of the project.

Site D (Eastern By-Pass river): is a stream that runs through latitude 04^0 47.79'N and longitude 07^0 0.89'E with 15.85m elevation above sea level. An abattoir/market is located along its bank. Effluents from the abattoir are washed in the river. Municipal refuse were also disposed into the river.

Site E (Ede street water-side): can be found on latitude 04^0 48.2'N and longitude 07^0 0.36'E with an elevation of 13.41m above sea level. The river that formed this site is highly polluted with organic waste from municipal waste and human feaces. Toilets built with stilts could be sighted on the river.

Site F (Nwaja river): located on latitude 04^0 48.56'N and longitude 07^0 0.93'E and 10.67m above sea level. The river is polluted with organic wastes from domestic waste disposal and human waste (feaces). The surrounding area is a shanty village/ market where traders live in their makeshift shop/shanty houses.

Collection of Insects

A standard dipping method described by Service, 1971, was used to sample the six aquatic habitats located in two local government areas for Dipteran larvae. In summary, 400ml plastic dipper was used to collect water from randomly selected points on the river. Dipping was done in five replicates and the contents sieved in a filter net. The larvae collected were sorted according to their families; a few were selected amongst the families to be reared to adults for further identification and confirmation while others were preserved in 80% ethanol. Collection for dipteran larvae was done bi-monthly from June 2011 to May 2012 between 8am to 12 noon.

Identification of Insects

The larvae and adults were identified at the Department of Animal and Environmental Biology, University of Port Harcourt using identification keys by Mendes (2011) and already identified and preserved insect types in the Entomological Research Laboratory of the Department.

Data Analysis

Data generated were subjected to Chi-square goodness of fit test using computer SPSS 16.0 windows application; similarity between the sites was determined using Jaccard's similarity index in PAST Version 1.99 windows application.

III. Results

Overall Abundance of Identified dipteran vectors.

Figure 1 shows the abundance of identified larvae of dipteran vectors observed during the study. A total of 1305 larvae and adult stages of Dipteran vectors consisting of 7 species were recorded in all the six sites. Of these, 28.28% were *Anopheles gambiae* s.l. comprising of 369 individuals , 11.03% were *Musca domestica* (144 individuals) , 39.54% were *Culex quinquefasciatus* (516 individuals), 3.45%, 3.45% were *Stratiomys longicornis* (45 individuals), 1.99% were *Ablabesmyia* sp(26 individuals), while 9.04% were *Sarcophaga haemorrhoidalis* (118 individuals) and 6.67% were *Chrysomya bezziana* (87 individuals) respectively.

Comparative abundance of dipterans across sampled sites.

Fig 2 depicts the comparative abundance of dipteran vectors in all the sampled sites. *Cx. quinquefasciatus* was most abundant in site A while the *Chrysomya bezziana* group was least abundant. *Sarcophaga haemorrhoidalis* was not observed in this site. Site B had *Musca domestica* as the most abundant species; site C had Anopheles followed by *Cx. quinquefasciatus* as most abundant species while in site D and E, *S. haemorrhoidalis* had the highest number but no species was dominant. However, site F had *Anopheles gambiae* s.l. as the most abundant species.

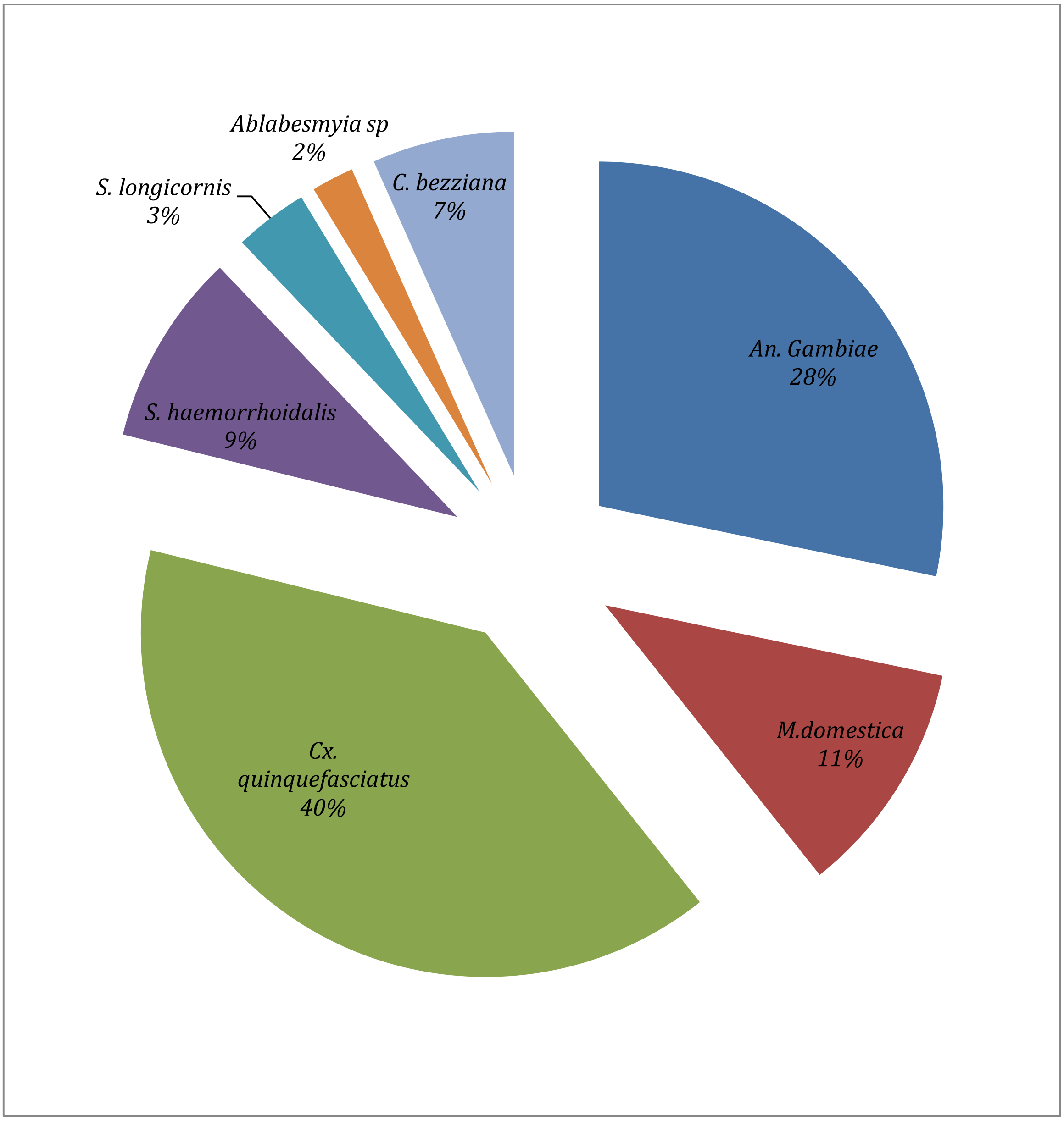

Figure 1: Overall Abundance of identified dipteran vectors across sampled sites.

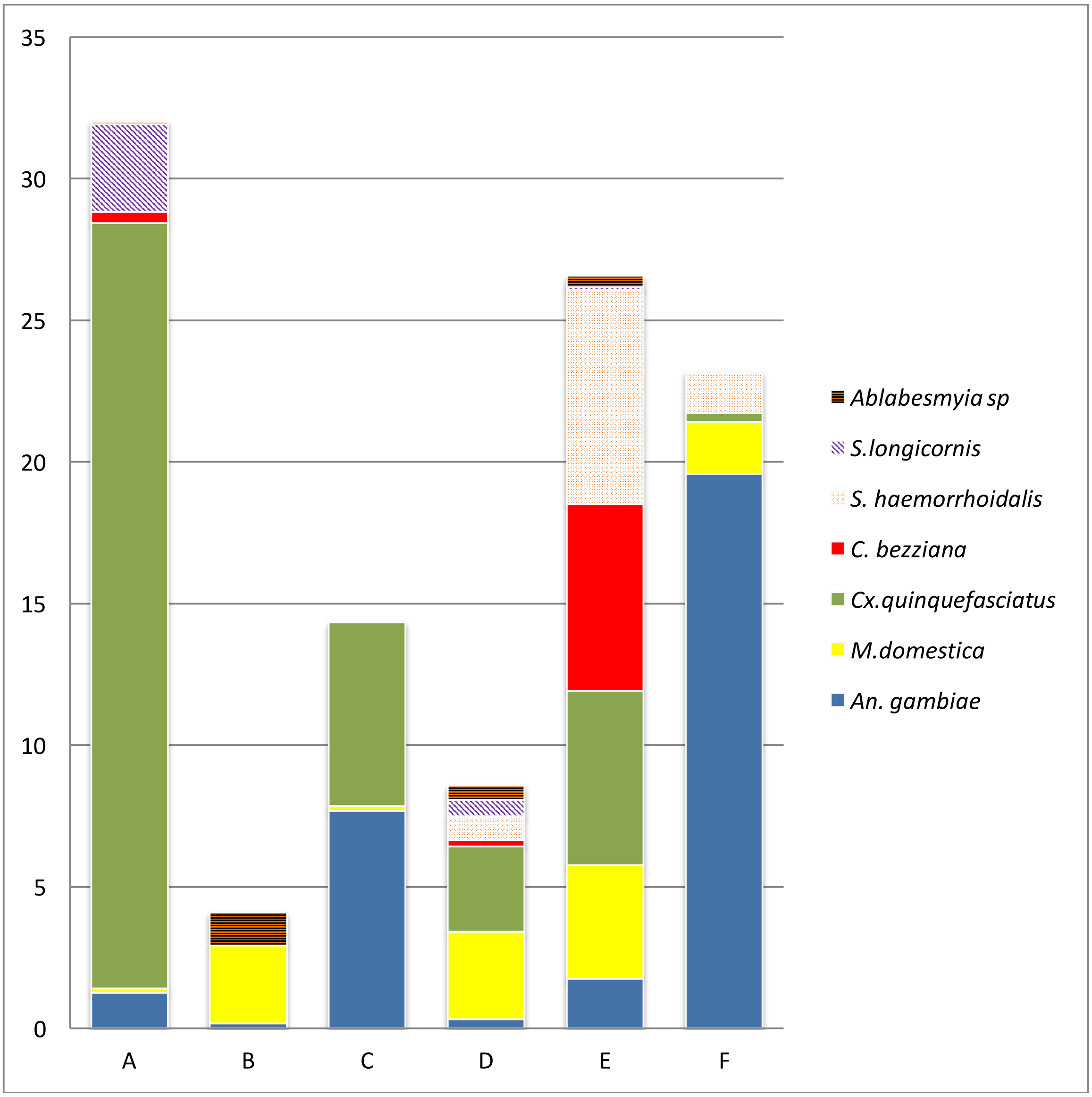

Figure 2: Comparative abundance of dipterans across sampled sites.

Monthly distribution of dipterans across the sites

Fig 3 shows the monthly distribution of dipteran flies at site A over the one year period of the work. *An. gambiae* s.l. was observed in the months of June, August, October, January and February, *M. domestica* was observed in June and April; *Cx. quinquefasciatus* was observed in June, January, March, April and May, with very high counts in January and May; the species *S. longicornis* was observed only in December while *C. bezziana* was observed in December and January. The other species were not found at this site.

At site B (Fig 4), *M. domestica* was observed only in December with 38 individuals, it was the most abundant vector at the site. *Ablabesmyia* sp was observed in May with 14 individuals while *An. gambiae* s.l., the least occurring was observed in September with just 2 individuals. This was the control site.

Fig 5 shows *An. gambiae* s.l occurring in June, August, November and January with its highest occurrence in November, *Cx. quinquefasciatus* was the most abundant vector at this site being observed in June, July, December and January and having the highest count (57 individuals) in July; *M. domestica* was the least abundant being observed in June and February with 1 individual respectively.

At site D (Fig 6) all the families were represented with *Cx. quinquefasciatus* being most abundant and *C. bezziana*, the least abundant. In all the months, vectors counts were made at this site with August having the highest count.

At site E (Fig 7) *Cx. quinquefasciatus* was observed in 6 months of the year (June, July, September, December, January and March) with the most count occurring in July. All other families were represented with varying percentages as shown in the graph.

In site F the highest number of vectors was recorded for one species (*Anopheles gambiae* s.l) numbering 200 individuals in July. *M. domestica*, *Cx. quinquefasciatus* and *S. haemorrhoidalis* were observed in varying percentages over the months.

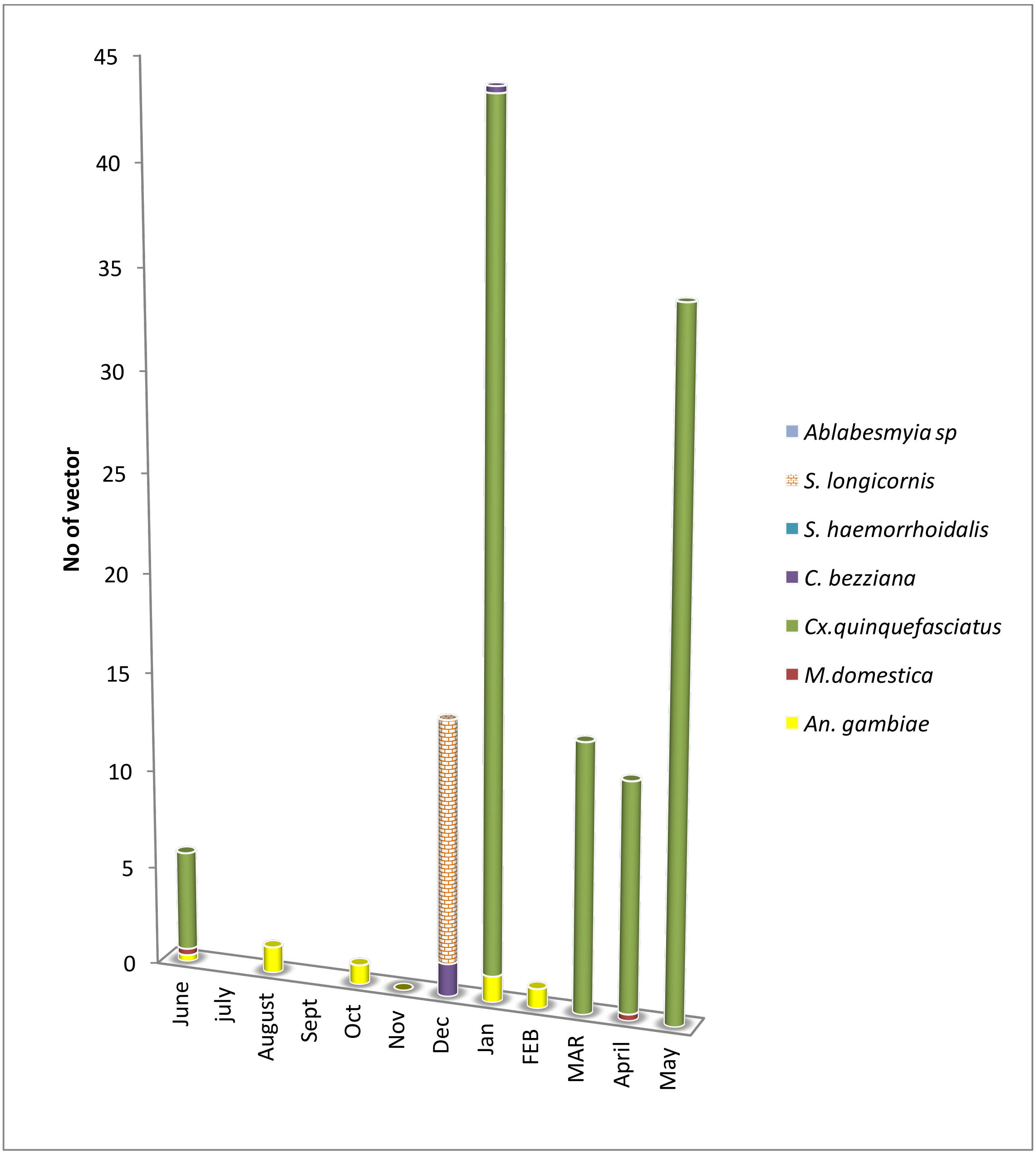

Fig 3: Monthly distribution of Dipteran vectors in site A (Rumuokoro river).

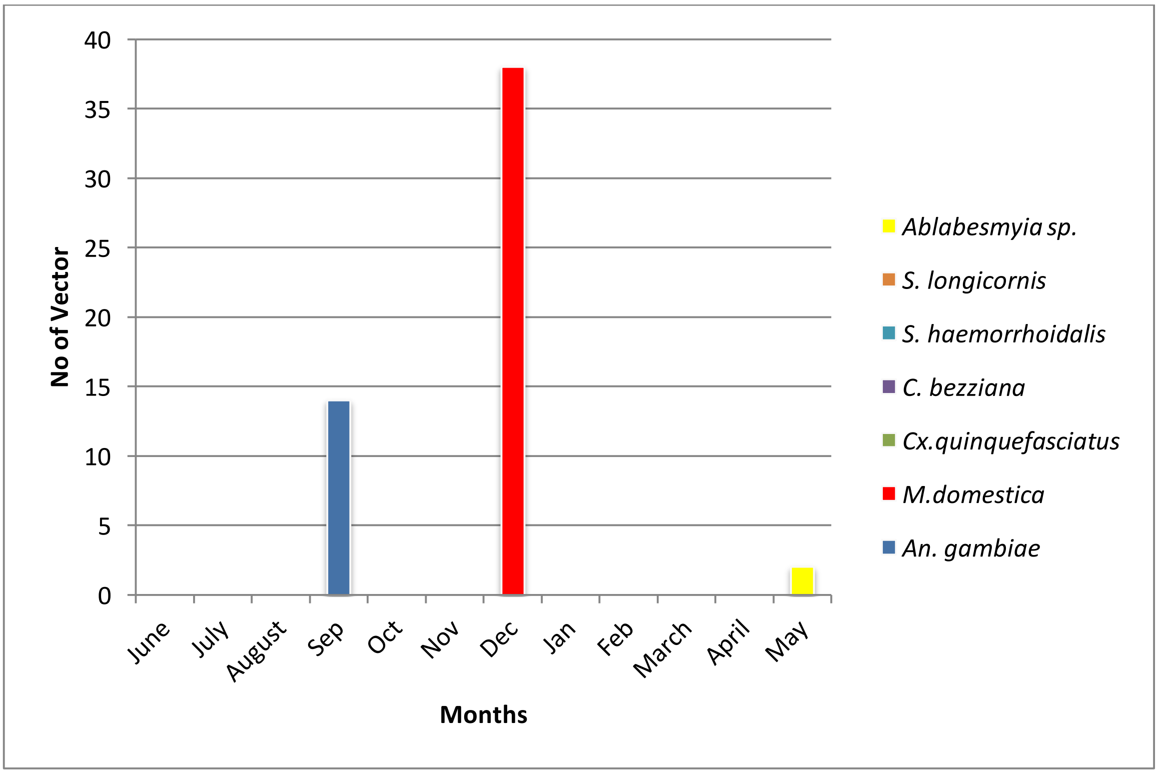

Fig 4: Monthly distribution of dipteran vectors at site B (Rumuolariver).

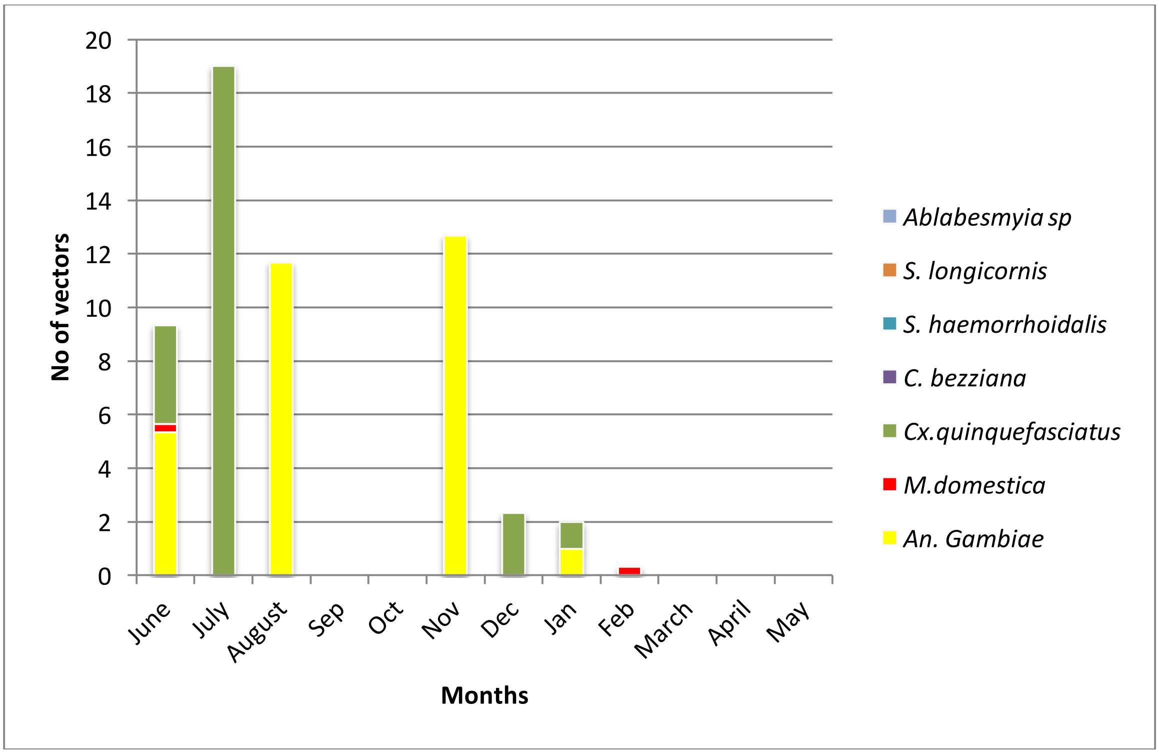

Fig 5: Monthly distribution of dipteran vectors in Site C (Ntanwogbariver).

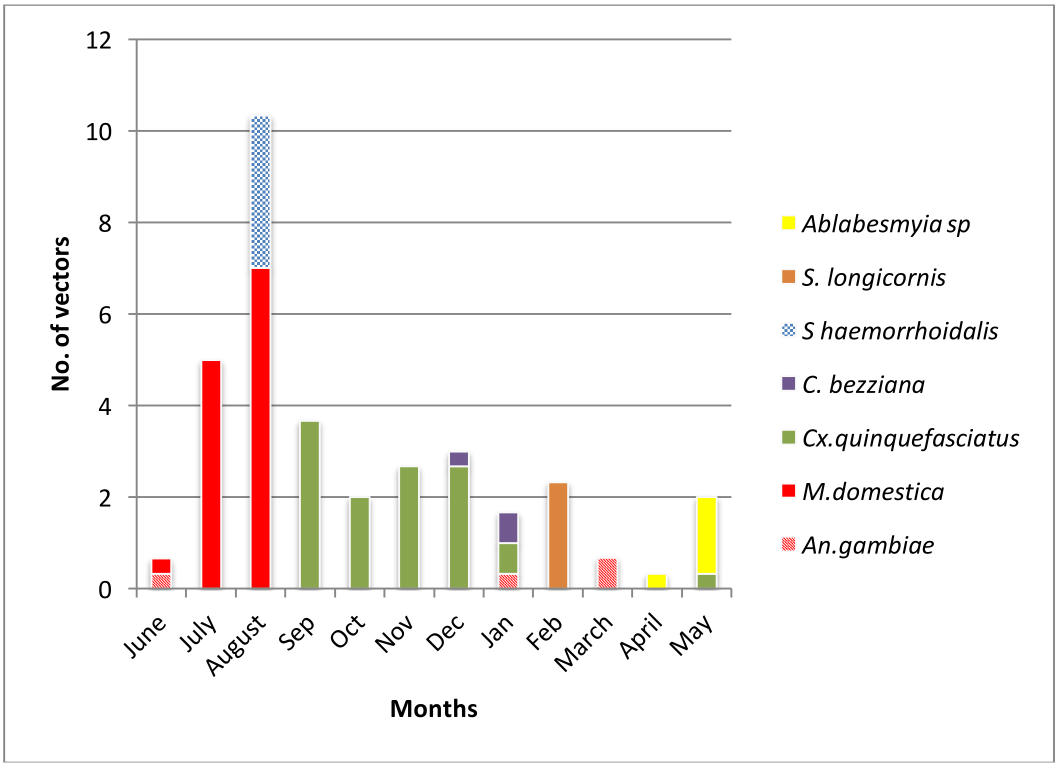

Figure 6: Monthly distribution of dipteran vectors in Site D (Eastern-By Pass River).

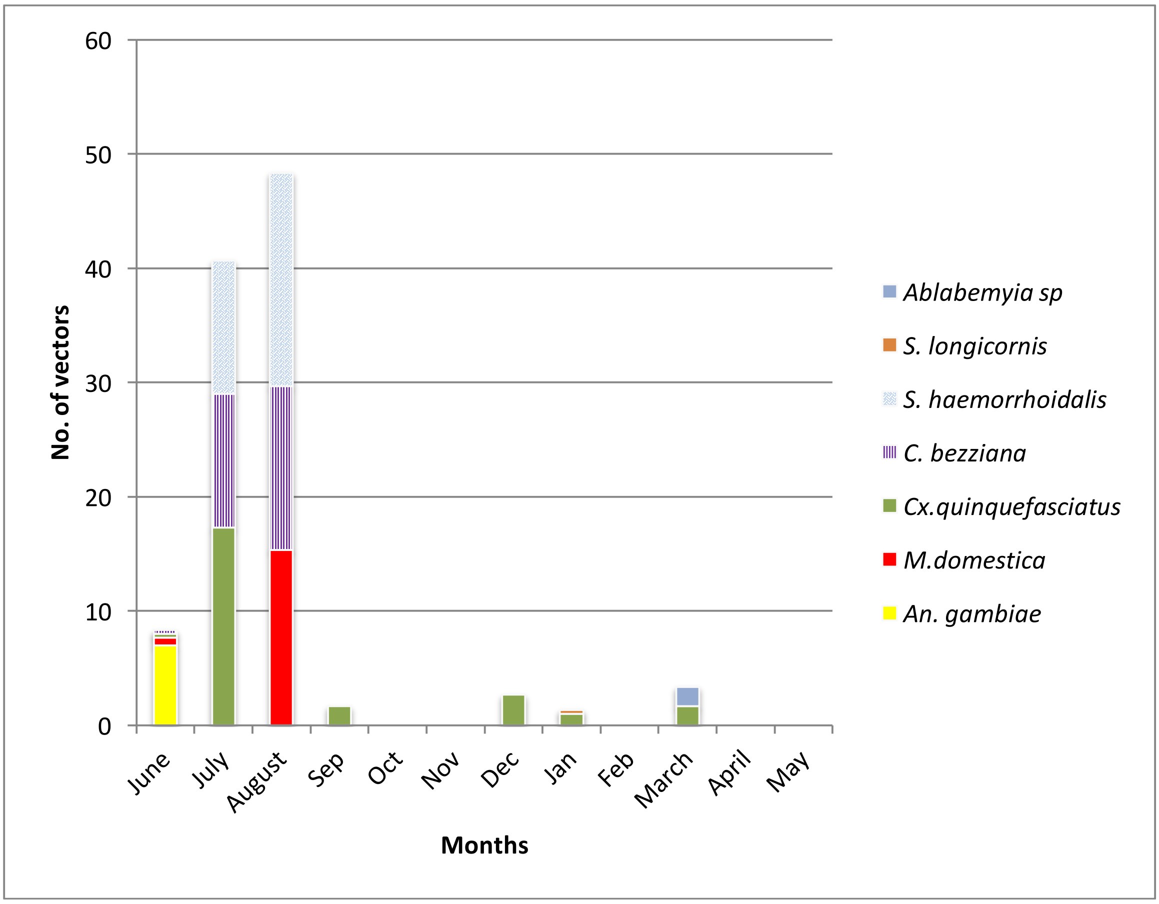

Figure 7: Monthly distribution of dipteran vectors in site E (Ede Water-side).

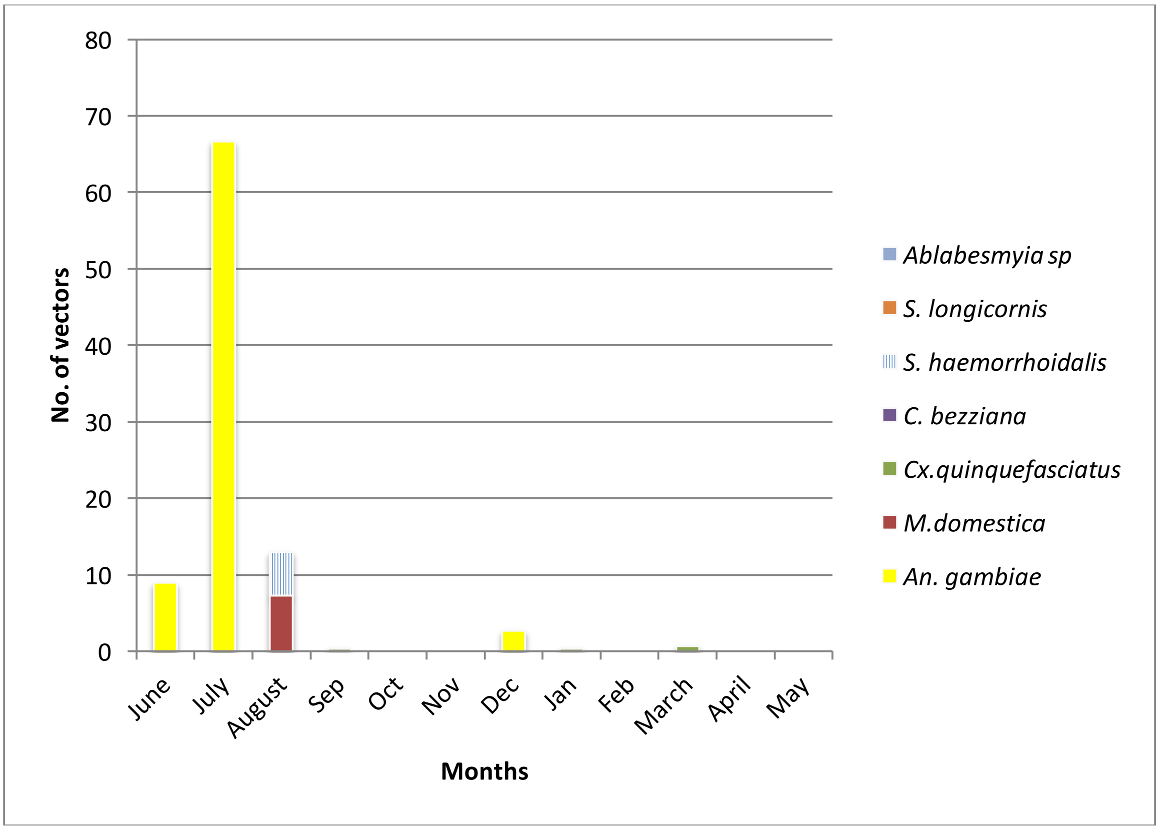

Figure 8: Monthly distribution of dipterans in site F (Nwajariver).

Comparison of dipterans diversity and abundance across sites.

Table 1 shows the result of Chi Square Goodness of fit Test, that was used to determine if there was significant difference in the abundance of vectors recovered from the various sites, a highly significant difference was observed (P<0.01). *Aposteriori* test further revealed that the abundance of dipteran vectors at site A was significantly higher than the abundance at other sites; site E and site F had no significant differences among themselves but were higher than sites B,C, and D (C>D>B).

For each select organism, as shown in the table below, significant differences were observed at different sites. In order of decreasing magnitude, *Anopheles gambiae*: sites (F>C>E>=A>D>B); *M. domestica*: sites (E=B=D>F>A=C); *Cx. quinquefasciatus*: sites (A>E=C>D>F>B); *S. haemorrhoidalis*: sites (E>F=D>A=B=C); *C. bezziana*: sites (E>D>A=B=C=F)

Table 1:**Comparison of means of abundance of dipteran vectors in the selected water bodies**

Taxa/ Sites	*An. gambiae*	*M. domestica*	*Cx. quinquefasciatus*	*S. haemorrhoidalis*	*S. longicornis*	CHI-SQUARE	P-Value
A	1.25^{c} (±0.49)	0.16^{c} (±0.11)	27.00^{a} (±12.85)	0.00^{c} (±0.00)	0.00^{c}		P<0.05
B	0.17^{e} (± 0.13)	2.75^{a} (± 2.75)	0.00^{e} (±0.00)	0.00^{c} (±0.00)	4.00^{a}		P<0.05
C	7.67^{b} (±4.12)	0.17^{c} (± 0.11)	6.50^{b} (±4.70)	0.00^{c} (±0.00)	6.25^{b}		P<0.05
D	0.33^{d} (± 0.19)	3.08^{a} (± 2.05)	3.00^{c} (±1.09)	0.83^{b} (±0.83)	7.58^{a}		P<0.05
E	1.75^{c} (±1.75)	4.00^{a} (± 3.82)	6.25^{b} (±4.24)	7.58^{a} (±5.27)	6.58^{a}		P<0.05
F	19.58^{a} (± 16.56)	1.83^{b} (± 1.76)	0.25^{d} (±0.35)	1.42^{b} (±)	320^{b}		P<0.05

IV. Discussion

Seven species of dipteran vectors were found associated with the selected water bodies with *Culex quinquefasciatus* being the most abundant (39.54%). This concurs with literature on this species that disposal of waste in opens drains constitutes an ideal breeding place for *Cx. qiunquefasciatus*, a known vector of Bancroftian filariasis (Braks*et al.,* 2007). Studies have also shown that oviposition of *Cx. quinquefasciatus* eggs is additionally enhanced in habitats containing water from combined sewage overflow which was similar to the conditions observed at the sites where this species was found (Calhoun *et al*., 2007). As was observed in the sampled sites with the exception of the control site, anthropogenic activities played a major role in creating suitable habitat for the spread of these disease vectors. However studies have also shown that the presence of the egg rafts of *Cx. quinquefasciatus* varies seasonally as was observed in the study (Chaves *et al.,* 2009*)*.

Anopheles gambiae was the second most abundant species (28.28%), this is unexpected since this species is usually found in containers containing clean water. However previous studies have shown that *An. gambiae* is demonstrating a worrying trend of adaptation to polluted waters in urban environments (Awolola*et al.,* 2007). This is because in recent years, the species has been found breeding in highly-polluted water sources in Cote d'Ivoire and Cameroon. In Lagos, Nigeria, the larvae of *An. gambiae* s.l. have been found in water sources with high concentrations of heavy metals such as iron, copper and lead, and other contaminants such as human feaces and petrol (Klinkenberg *et al.,* 2008). These findings suggest that the pollution associated with urbanization will increase vector densities in Nigerian cities and that urban vector control will become increasingly relevant in few years to come. It is noteworthy however, that poverty, deteriorating infrastructure and overcrowding are some of the factors that contribute to the development of conditions that modify Anopheline breeding sites. As more people move into cities and industrialization proceeds, urban malaria will be on the increase in Nigeria.

The literature on *Ablabesmyia sp* (Chironomidae) agrees with the observations on this species in this study. Previous literature suggests that they occur in ponds, lakes and sewage systems. Large chironomid emergence from polluted bodies of water are common and may cause local annoyance to humans in addition to economic damage to machinery, paint finishes, automobiles and airplanes (Ali, 1991). Several serogroups of *Vibro cholerae*, the bacterium responsible for cholera, have been isolated from chironomid egg masses and from the cuticle of adults. This suggests that they may be involved in the maintenance and movement of *V. cholera* in and between bodies of water (Broza *et al*., 2005). Large numbers of adult chironomid can discourage tourism and cause contamination of materials in food processing, pharmaceutical, and manufacturing plants. Larvae that find their way into water-storage and water distribution systems can pass through taps into homes (Bay, 1993). In this study however, *Ablabesmyia* sp. was the least abundant species, whereby only 26 individuals constituting 1.99% of the total dipteran species encountered in the study. Its presence however, is indicative of the unhealthy nature of these water bodies.

Larvae of *Stratiomys longicornis (*Stratimyidae) were found primarily in the sites that housed and featured human feaces. This agrees with earlier documented findings on this species that they breed in animal wastes, decaying plants and human wastes. Larvae of this dipteran fly are occasionally eaten by humans in overripe fruits or undercooked meat and sometimes result in intestinal myiasis (James, 1947).

Musca domestica larvae with 144 individuals were the third most abundant dipteran vector observed in this study, constituting 11.1% of the total larvae recovered. The sanitary condition of the sites where this species was observed corresponds with literature on the insect. Major breeding sites of *M. domestica* according to Mullen and Durden, (2009) include human garbage dumps, open privies, livestock manure, soiled beddings, poultry litter, and wastes around fruit and vegetable processing plants. From a public health stand point, *M. domestica* is probably the most significant as a nuisance and potential vector of enteric pathogens.

The larvae of *Chrysomya bezziana* was found in three sites associated with carrion and this is supported by previous findings on this species. The importance of this species as a primary agent of cutaneous myiasis and its impact on animal production is well known (James, 1947).

Sarcophaga haemorrhoidalis was found in sites with human feaces as earlier reported by Mullen and Durden, (2009) who observed that this species are associated with carrion or feaces, and cause facultative wounds and accidental gastrointestinal myiasis.

The abundance of *Anopheles gambiae* s.l. was found to have peaked in the month of July, at site F (Nwaja), while the least abundance was observed in September and March. Site F is characterized by organic wastes from domestic waste disposal. *An. gambiae* was not observed in April and May 2012. Typically, at the beginning of the long or during the short rains, the number of *An. gambiae* becomes predominant, while at the end of the rains the density declines (Kigadye, *et al.,* 2010).

Culex quinquefasciatus was most abundant in January while few of the mosquitoes were recorded in October. The species was not recorded in August and February. This corresponds with findings by Murty *et al*., (2002). The species occurred significantly in sites B, D, E and F, having most abundance in August 2011 with least abundance in February and April. *Musca domestica* was not recorded from September – November, and January, March and May. This finding corresponds with the findings by Bong and Zairi, (2009).

Ablabesmyia sp occurring mostly at the control site (site B). The species was the least abundant of all identified dipterans. This is an indicator species of organic pollution; its presence at the control is indicative of the health status of a water body (Bay, 1993).

V. Conclusion

This study provides information on the composition, distribution and diversity of Dipterans in the water bodies studied. It also provides information on the monthly variation in the relative abundance of the Dipterans in the sampled water bodies. It can serve as a reference point for future studies on these rivers and other rivers worldwide, providing information of the type of insect species associated with water bodies with organic pollution.

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