CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria is a vector-borne disease caused by protozoan parasites of the genus *Plasmodium* (Family: Plasmodiidae). The major species of the parasites that cause malaria in man are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. There is also increasing recognition of enzoonotic transmission of a simian species, *P. knowlesi* to man. However, the focus of enhanced research on malaria control has primarily been on *P. falciparum*, given the mortality and severity of the disease state associated with this species. Approximately half of the world populations living in about one hundred and nine countries were at risk of contracting this serious and often life-threatening disease (Targett and Greenwood, 2008). Malaria has accounted for approximately 250 million cases and nearly one million death yearly, the great majority of which occured in children under 5 years of age (Hall and Fauci, 2009; Corradin and Kajava, 2010). About 90% of deaths in Africa were mainly due to malaria and this was more prevalent in children below 5 years of age (WHO and UNICEF, 2003). Malaria has accounted for 10% of the continent's overall disease burden, 40% of public health expenditure, 30-50% of in-patient hospital admissions and up to 50% of outpatient visits in areas with high transmission (WHO, 2006).

According to WHO (2015), about 3.2 billion people were at risk of malaria. It was also reported that there were 214 million global cases of malaria, with the African region accounting for 88%, followed by the South East Asian region with 10% and the East Mediterranean region with 2%. Depending on the degree of transmission in the areas, malaria risk can be stratified into categories such as malaria free, unstable (epidemic) or stable (endemic). In endemic areas, the adult population usually shows a high level of immunity to malaria and children are therefore more often at risk of severe disease and death due to malaria than are adults. Furthermore, endemicity of malaria can be classified based on parasite rate as hypoendemic (< 10% prevalence rate), mesoendemic (11-50% prevalence rate), hyperendemic (51-75% prevalence rate) and holoendemic (> 75% prevalence rate) (WHO, 2003).

In Nigeria, the risk of malaria infection exists throughout the country. It is endemic in Anambra State with the prevalence of 76% in Azia (Aribodor *et al*, 2003), 46% in Nnewi (Umeanaeto *et al.,* 2006), 67% in Umudioka (Onyido *et al*., 2010), 58.2% reported in Ogbunike (Onyido *et al.*, 2011), and 46.3% reported based on survey in thirteen communities of Anambra State (Okeke *et al*., 2016). Approximately 50% of the Nigerian population experience at least one episode per year. However, official estimate suggests as much as four bouts per person per year on the average (WHO, 2003). In Nigeria, malaria is the number one public health problem (Onwujekwe *et al.*, 2000; FMH, 2001) and has been responsible for about 300,000 deaths every year (Coker *et al.*, 2001; WHO, 2003). Malaria accounted for 40% public health expenditure (USAID Health, 2005) and the cost of malaria treatment and prevention in Nigeria was estimated to be over $1 billion per annum (Odaibo, 2006).

Malaria parasites are solely transmitted in nature through the bites of infected female anopheline mosquitoes. There are more than 400 *Anopheles* species world-wide, but only about 70 of them are capable of transmitting malaria parasites under natural conditions, and approximately 40 species are considered to be major vectors of importance (Service and Townson, 2002). In Africa, South of Sahara, the main vectors of malaria belong to the members of *An. gambiae* s. l. and *An. funestus*. *An. gambiae* s. l. comprises seven genetically distinct sibling species that can be identified on a cytogenetic basis or using molecular techniques (Coluzzi *et al*., 2002). They include *An. gambiae* s. s., *An. arabiensis*, *An. quadriannulatus* Theobald species A and B, *An. bwambae* White, *An. melas* Theobald and *An. merus* Donitz. The secondary vectors include *An. moucheti* and *An. nili* (Molineaux and Gramiccia, 1980; Gilles and Coetzee, 1987). *An. pharoensis* is also widely distributed in Africa and can maintain active transmission of malaria even in the absence of the main malaria vectors (Janssens and Wery, 1987). *An. pretoriensis*, *An. tenebrosus* and *An. rhodesiensis* were documented in SouthWest Ethiopia (Abraham *et al*., 2017). A large number of *Anopheles* species have been reported in Nigeria, but the main vectors of malaria belong to the members of *An. gambiae* s. l and *An. funestus*. The secondary vectors include *An. moucheti* and *An. nili* (Molineaux and Gramiccia, 1980; Gilles and Coetzee, 1987). In other malaria endemic areas, different *Anopheles* species are responsible for malaria transmission.

Anophelines like all other mosquitoes have four stages in their life cycle: egg, larvae, pupae and adult. The first three stages are aquatic, lasting for 5 to 14 days depending on the temperature (Service and Townson, 2002). The adult male and female *Anopheles* species thrive on nectar and sugary substances for their nutrient requirements; but adult females also feed on blood. An adult female *Anopheles* mosquito normally mates only once in her lifetime and she requires the blood meal after mating for the eggs to develop. Blood meals are generally taken every two to three days; before the next batch of eggs is laid. In the course of that, they may pick up malaria parasites when they bite an already infected host. The *Anopheles* mosquitoes can therefore carry infective sporozoites in their salivary glands, which they transfer to the blood stream of a susceptible host during another blood meal (Najera and Hempel, 2006). In some parts of Africa that are highly endemic, a person may receive one infective bite every night (WHO, 2003).

As a result of the medical importance of *Anopheles* mosquitoes, World Health Organization recommended vector control as an important component of the global strategy for preventing malaria. This includes the use of insecticide treated nets (ITN) and application of indoor residual spray (IRS). ITN is known to kill mosquitoes and also have proven repellent properties that reduce the number of mosquitoes that enter the house (Curtis *et al.*, 2003). It has been found to reduce clinical malaria by over 50% and mortality in children aged 0-59 months by 15-30% when the overall population coverage is greater than 70% (Choi *et al.*, 1995; Eisele *et al.*, 2003). Also ITN has been shown to be effective in the control of malaria even when the level of usage is low (Egbuche, *et al.*, 2013). Thus the efficacy and cost-effectiveness of Insecticide-Treated Net (ITN) in reducing malaria related morbidity and mortality is well-known (Goodman *et al.*, 1999; Lengeler, 2000) and has led to massive distribution of millions of free or highly subsidized ITN to vulnerable populations in sub-Saharan Africa (Guyatt *et al.*, 2002; Grabowsky*, et al.,* 2005; Eisele *et al.*, 2006; Thwing *et al.*, 2008). In some areas, malaria vectors that rest indoors can be prevented or controlled by spraying the inside of houses with a residual insecticide. This reduces the longevity of mosquitoes below the time it takes for the malaria sporozoites to develop; thus reducing the density of indoor resting and biting mosquitoes. IRS at 85% coverage of target risk populations and target structures, remain the most efficacious and effective malaria control intervention to reduce malaria transmission rapidly and reduce malaria morbidity and

mortality by 50% at an affordable cost (National Malaria and Vector Control Programme, 2010). Elimination of locally important malaria vectors can also occur though indoor residual spraying.

1.2 Statement of problem

The pattern of malaria parasites transmission is largely a function of the *Anopheles* mosquito breeding ecology (Greenwood *et al.*, 2008) and the proximity of human dwelling places to vector breeding sites among others (Onyido *et al.*, 2009a). *An. gambiae* s. l., the principal transmitter of malaria in Nigeria is closely associated with sunlit water collections close to human dwellings. On the contrary, *An. funestus* which is another important malaria vector in Nigeria tends to breed more in cool, clear, shaded, permanent water bodies relatively undisturbed by man in rural areas (Onyido *et al.*, 2009b). Though *Anopheles* species are known to be ground pool breeders, a large number of them have been observed in clean collections of water in gutters and domestic containers (Mafiana *et al.*, 1998; Aigbodion and Odiachi, 2003). *Anopheles* mosquitoes have also been found to breed in clear water of suitable pH, temperature and nutrient composition (Okorie, 1978). They can also be found in fresh or salt water marshes, mangrove swamps, rice fields and grassy ditches as well as the edge of streams and rivers.

Other behavioural changes in the breeding ecology of *Anopheles* species have equally been reported. They have been found to breed in polluted water bodies containing heavy metals (Awolola *et al*., 2007) and high level of organic materials (Sattler *et al*., 2005). A few species were reported to breed in tree holes or the leaf axils of some plants (CDC, 2004; Omlin *et al*., 2007). These are deviations from the earlier knowledge that *Anopheles* species breed in clean pool of water and this could mean an expansion in the range of breeding habitats utilized by them.

According to Autino *et al*. (2012), the degree of endemicity of malaria in any region is determined by species of indigenous anopheline mosquitoes, their relative abundance, feeding, resting behaviour and their individual suitability as hosts for *Plasmodium* species, among others. Adult female *Anopheles* mosquitoes differ generally in their behaviour especially in biting habits, host blood meal preferences, time and place of biting and resting sites. All these are very

important in the malaria transmission. Some species are strictly zoophilic (feeding on animals only), or strictly anthropophilic (biting only humans), while others bite both man and animals indiscriminately. Also some bite and rest indoors (endophagic and endophilic) but others bite and rest outdoors (exophagic and exophilic). Furthermore, some are crepuscular and nocturnal and others are diurnal (Suárez-Mutis *et al*., 2009).

In addition, vector control interventions that target indoor biting and resting mosquitoes can cause changes in *Anopheles* species ecology thereby influencing the transmission of malaria. These mosquitoes have become very successful in spreading of malaria, as they have over the years devised means and mechanisms of adapting to environmental conditions. Their behavioral adaptations are a serious impediment to the success of malaria control measures in place. They include change in both, the peak biting time as well as outdoor and indoor feeding preference due to application of control measures (Fornadel *et al*., 2010, Moiroux *et al*., 2012, Thomsen *et al*., 2017). Their feeding behaviours are likely to have changed to maximize available feeding opportunities with human exposure to mosquito bites translating into enhanced malaria transmission. For instance, the effectiveness of insecticide treated mosquito nets varies with the pattern and rate of malaria transmission. The nets do not work well in many areas of low and unstable transmission, where malaria vectors bite in the early evening and morning (Kroeger *et al.*, 1999) as well as in outdoor locations. There is also shift from endophily to exophily in certain population of *Anopheles* mosquitoes as a result of Indoor residual spraying (Pates and Curtis, 2005). Generally, endophilic mosquito populations may include varieties that exhibit exophilic tendencies.

More so, there is large site variation in the spatio-temporal dynamics of malaria vector populations indicating that the risk of malaria parasites transmission differs among sites (Ndenga *et al*., 2006). The differences in malaria risk among the sites can be explained by vector species of local importance, availability of breeding habitats, preferred host, and environmental conditions among others (Imbahale *et al*., 2011; Atieli *et al*., 2011).

1.3 Justification for the study

Entomological measures of transmission are important metrics that can be used to provide a better understanding for the control of malaria. For this to be successful, good knowledge of the breeding ecology of *Anopheles* species including the types and preference for larval habitat, spatial and temporal distribution of breeding sites as well as the physical, biological and chemical characteristics of the habitats are required (Olayemi *et al.*, 2010). The metrics also require identifying and testing a large number of mosquitoes for host preference and infectivity. In addition, malaria parasite prevalence and intensity studies are also required to confirm the endemicity level and impact of malaria control in a given location.

In Nigeria and Anambra State, a lot of research has been carried out on malaria. However, most data sets are cross-sectional, which restricts their use for determining transmission efficiency. This is because parameters determining sporozoite prevalence are likely to vary seasonally from location to location. The productivity of different breeding sites across the seasons is not well documented in Nigeria as several intermittent studies have focused on mosquito abundance and distribution. In addition, no host selection studies have been conducted in the study area. These *Anopheles* species and their complexes that vary in behaviour and vectoral capacity present a real problem to malaria control (Hougard *et al.*, 2002). Many of these problems can be overcome using longitudinal studies, where the biology and epidemiology of the parasite and vector are likely to be more consistent.

Thus, for malaria control measures to be successful in Anambra State, good knowledge of the dynamics of malaria transmission is important as it provides insight into the magnitude of the problem and helps to define when and where the greatest malaria risk occurs. Anambra East LGA was primarily chosen for this study because of large bodies of water surrounding it which may provide potential breeding sites for mosquitoes throughout the year; as aquatic habitats are an important component in malaria transmission. According to Gillies and Coetzee (1987), malaria vectors are known to breed in open sun-lit pools of water and relatively large permanent water bodies with vegetation. Secondly, malaria transmission is geographically specific and studies by Okwa *et al.* (2009) demonstrated the complex distribution of anopheline mosquitoes

and considerable variations in the intensity of malaria transmission in four ecological zones of Nigeria. A lot of research on malaria and malaria vectors have been carried out in different areas of Anambra State but there are very scanty data on Anambra East LGA. Also, malaria vector ecology has not been studied in depth using longitudinal research design in Anambra State and the breeding habitats of *Anopheles* mosquitoes have not been characterized in Anambra East LGA. It is well known that various species of the genus *Anopheles* play unequal roles in the transmission of human malaria parasites. In given areas, some *Anopheles* species are either the major vectors, secondary vectors or are not involved in malaria transmission at all because of their occurrence, abundance and interrelations with humans (Artemiev, 2001). Therefore, there is also need to identify the species present in Anambra East LGA and their vectoral capacities which have not been studied.

1.4 Aim and objectives

The aim of this research was to investigate the ecology of malaria vectors and the endemicity of malaria in Anambra East Local Government Area of Anambra State.

The specific objectives were to determine:

1. Malaria vector species composition and the climatic factors influencing their survival and population abundance.
2. Breeding ecology, physicochemical and biological factors operating in *Anopheles* mosquito breeding habitats.
3. Biting and resting behavior of the adult *Anopheles* mosquitoes to identify their preferred biting time, biting location and resting location.
4. Entomological indices (Human Blood Index, Sporozoite Rate and Entomological Inoculation Rate of *Anopheles* species) of malaria transmission in the study area.
5. Malaria endemicity through prevalence / intensity studies of malaria in the study area with respect to age, gender, month and seasons of the year.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria vector species composition and the climatic factors influencing their survival and population abundance.

2.1.1 *Anopheles* species composition and distribution in Africa

In Africa, *An. arabiensis*, *An. coluzzii* and *An. gambiae* s. s. from the *An. g*ambiae s. l. and *An. funestus* from the *An. funestus* subgroup are the most important vectors transmitting malaria parasites to humans (Battle *et al*., 2012; Sinka *et al*., 2012; Coetzee *et al*., 2013). Also within the *An. gambiae* s. l., *An. melas* and *An. merus* are considered dominant vectors in at least one region (Kipyab *et al*., 2013; Ebenezar *et al*., 2016). *An. gambiae* s. s., *An. funestus, An. arabiensis and An. moucheti* are the common species of mosquitoes that transmit malaria parasites in Nigeria (Okorie *et al*., 2011). The main malaria vectors in the urban areas of Southern Ghana is *An. gambiae* s. l.; with *An. coluzzii* and *An. gambiae s. s* as the most abundant sibling species identified (Dzorgbe *et al*., 2017). Over 99% of the 5,802 morphologically identified *Anopheles* species in Accra and Sekondi-Takoradi Metropolitan Areas of Ghana were *An. gambiae* s. l of which more than 99% of the studied 898 individuals were *An. coluzzii* (62%), *An. gambiae* s. s. (34%) and *An. melas* (0.20%). Other species identified included *An. rufipes* and *An. coustani* (Mattah *et al*., 2017). *An. coluzzii* were reported to be more prevalent in savannah areas as compared to *An. gambiae* s. s. which was more dominant in forest zones (della Torres *et al*., 2005; de Souza *et al*., 2010; Hunt *et al*., 2011; de Souza *et al*., 2013). In Niger Republic, three members of the *An. gambiae* complex were found: *An. arabiensis*, *An. coluzzii*, and *An. gambiae* s. s. (Labbo *et al*., 2016). In Kenya, the three species of *Anopheles* that are the most abundant, and which have the highest rates of transmitting malaria, were *An. funestus, An. gambiae*, and *An. arabiensis*. *An. gambiae* dominated the highland areas whereas *An. funestus* dominated the low land areas (Ndenga *et al.*, 2006). In Mozambique, *An. arabiensis* and *An. merus* were involved in malaria transmission (Mendis *et al*., 2000; Cuamba and Mendis, 2009). *An. funestus* was directly implicated in malaria transmission in South Africa (Hargreaves, *et al*., 2000).

2.1.2 Climatic factors influencing the survival and population abundance of *Anopheles* mosquitoes.

Climatic factors have been associated with mosquito abundance and transmission of mosquito borne infections in the tropical regions of the world. Changes in the pattern of temperature, rainfall and relative humidity may directly or indirectly influence the dynamics of malaria vector population and subsequently the spread of the disease (Wu *et al*., 2007). Among these climatic factors, rainfall is the main cause of temporal change in mosquito abundance. Rainfall provides breeding sites for mosquitoes to lay their eggs which develop to adult stage. Nevertheless, excessive rain can eliminate larval habitat through flooding, thereby decreasing the vector population especially *Anopheles* species because they require sunlit pool of water. Rainfall also ensures a suitable relative humidity of at least 50% - 60% to prolong mosquito survival. *An. gambiae* s. s. had the highest abundance during the rainy periods (May - August) (Simon-Oke and Olofintoye, 2015). Ambient temperature plays a major role in the life cycle of the malaria vectors. The rate of development from one immature stage of *An. gambiae* s. s. to the next increased at higher temperatures to a peak around 28ºC and then declined. Also, adult development rate was greatest between 28ºC and 32ºC, although adult emergence was highest between 22ºC and 26ºC (Bayoh and Lindsay, 2003). The gonotrophic cycle which is the time between blood meals of the vector is short at higher temperatures because digestion speed increases (Haque *et al*., 2010) and this will ensure an increase in the vector species abundance. A suitable relative humidity of at least 50% - 60% prolongs mosquito survival; relative humidity below 60% shortens the life span of the mosquito vectors (Rogers and Randolph, 2006). Thu *et al*., (1998), reported that temperature at 28ºC with 50% - 55% relative humidity is the most appropriate condition for the elevation in mosquito density or abundance. Similarly, Simon-Oke and Olofintoye (2015) reported that temperature range of 26ºC to 32ºC with average humidity of 55% facilitate the higher mosquito abundance.

2.2 Breeding ecology, physicochemical and biological factors operating in *Anopheles* mosquito habitat.

2.2.1 Breeding sites of *Anopheles* mosquitoes

Mosquitoes naturally breed in water. The immature stages (eggs, larvae and pupae) are aquatic while the adults are terrestrial. The survival and capacity to transmit diseases by mosquitoes are directly linked to the availability of breeding sites (Onyido *et al.*, 2006). Mosquitoes generally breed in various types of stagnant water bodies, both natural and artificial, at various seasons of the year. Mosquito larval stages develop in these larval habitats to emerge and become adults, ready to bite and transmit both human and animal diseases. However, adult mosquitoes show a very distinct preference for the types of water sources in which to lay their eggs. Different sizes of water collections provide suitable habitats for abundant and diverse anopheline mosquitoes that support efficient malaria transmission all year round (Adeleke *et al*., 2008; Ndenga *et al*., 2011). Generally, *An. gambiae* breed in sunlit stagnant water collections around our homes, streets, and streams or other quiescent water collections, from where they fly into the houses to bite man (Onyido *et al*., 2014). Some species breed only in freshwater; some prefer brackish water, some like standing water around human habitations such as puddles, broken coconut shells or trash that collects water (Robert and Janovay, 2009). Nevertheless, an *An. gambiae* female uses multiple breeding sites for oviposition (Chen *et al*., 2006). They are also well known to be very adaptable to increasing ecological and environmental changes because of their high level of genetic diversity and plasticity.

Increase in ecological and environmental modification due to agricultural activities and urbanization has been observed to contribute to the breeding of various mosquito species including *Anopheles* species (Adeleke *et al*., 2008). *Anopheles* species have been reported to adapt themselves to the various ecological circumstances provided by all stages of rice culture including nursery, watering, planting, growing, tillage, maturation, harvesting and land fallow (Onyido *et al*., 2014). *Anopheles* mosquitoes breeding in refuse dump have also been reported (Ezihe *et al*., 2017). In many areas of Africa, including Nigeria, *An. gambiae* which is the main vector of malaria parasites breeds in exposed, often muddy sunlit ground pools of water of

various sizes ranging from brick pits, animal footprints and vehicle tyre prints. It is occasionally found in man-made containers such as wheel barrows, mortar, pans, open tanks, canoes and abandoned concrete mixers (Onyido *et al*., 2014). *An. funestus* complex has uneven distribution throughout Nigeria. It breeds in cool shady fresh water swamps along rivers and streams and other water pools often associated with water lettuce, *Pistia stratiotes* and grasses at the edges of rivers. *An. melas* are essentially coastal species and a member of *An. gambiae* complex. It is a major vector of malaria especially around lagoons. *An. arabiensis* survives better under drier conditions in lowlands than *An. gambiae* s. s (White *et al*., 1972; Minakawa *et al*., 2002), and it has been found to dominate irrigated areas such as rice fields (Mwangi and Mukiama, 1992) among other habitats. Some oviposit in plant axils such as those formed by banana, pineapple, *Ravanella*, bromeliads, grass, and in cavities of pitcher plants (Service, 1970).

The location of *Anopheles* mosquito larvae in a habitat is due to selection of oviposition site by gravid females, and the numbers of habitats available for sampling depend upon larval dispersal and survival. The two principal vectors of malaria in this group, *An. gambiae* s. s. and *An. arabiensis*, are broadly sympatric but there are areas where only one or the other may be found (Levine *et al*., 2004). The abundance of *An. gambiae* s. s. and *An. arabiensis* larvae differed significantly among habitats (Edillo *et al*., 2002). Immatures of *An. gambiae* s. s. were more numerous than *An. arabiensis* among all sampling sites in rock pools, the swamp and puddles where they were sympatric (Edillo *et al.*, 2006). In laboratory a study, McCrae (1984) observed that *An. gambiae* s. l. laid more eggs in petri dishes with turbid water from a natural site than in distilled or tap water.

Availability and stability of these aquatic habitats are very crucial in determining year round productivity of malaria vectors (Himeidan *et al*., 2009). This is because some of the breeding habitats are permanent (stable) and some others are temporary as they tend to dry up or are cleared along side with refuse. Larval counts and density of *Anopheles* mosquitoes are known to be high during rainy seasons and decline during dry seasons (Lamidi, 2009; Oyewole *et al*., 2010; Donovan *et al*., 2012). This is obviously due to loss of some habitats and decline in mosquito populations during the dry season. Also habitat availability was significantly correlated

with the density of indoor resting mosquitoes in houses near to larval sites (Minakawa *et al*., 2005). Even among the available breeding habitats, malaria vectors are often present, abundant and their adults produced in large numbers in some habitats and not in others. This shows that the productivity of the breeding habitats may differ.

Minakawa *et al* (2005) found out that habitat stability and abundance of *An. gambiae* s. l. was positively correlated with habitat size. Smaller habitats normally have low abundance of mosquito larvae and are easily lost than bigger habitats. During the dry season, limited rainfall can create pools of water along the river bank, thereby providing good breeding site for *Anopheles* species, which favours disease transmission (Gubler *et al.*, 2001). In a dry season also, *An. gambiae s. l.* pupae were distributed between burrow pits and pools in streambeds (Mutuku *et al*., 2006) when the smaller habitats had dried up.

Habitat characteristics such as the physical chemical and biological characteristics of the habitats (Edillo *et al.*, 2006; Muturi *et al.*, 2007; Fillinger *et al.*, 2009; Robert *et al*, 1998 and Minakawa *et al.*, 2005) are important factors in determining habitat productivity. Changes in the physicochemical and biotic characteristics of surface water habitats may create conditions either favorable or unfavorable to the breeding success of mosquitoes, depending on the ranges of tolerance of different species (Chen *et al.,* 2006).

2.2.2 Physicochemical parameters of *Anopheles* mosquito breeding habitats

Mosquitoes prefer an environment with certain physical and chemical conditions in sufficient amount and at appropriate time for survival and development (Adeleke *et al*., 2008). According to Mutero *et al.* (2004) and Okorie (1978), mosquitoes show preference to water with suitable pH, optimum temperature, dissolved oxygen, concentration of ammonia, and nitrate. These physico-chemical parameters have been found to affect larval development and survival in breeding water. Ezihe *et al*. (2017) reported that temperature and Lead (Pb) showed strong positive correlation with mosquito larval abundance whereas Copper (Cu) showed a strong negative correlation. The physico-chemical parameters required also vary from one species to another. For instance, pH of 7.4 was found to be suitable for *Aedes* mosquitoes (Adebote *et al*.,

2008; Afolabi *et al*., 2010). Similarly, the work of Okogun (2005) established that water of a near neutral pH 6.8 to 7.2 was found most optimal for the weakening of the egg shells for the first instars larval stage to emerge. Adebote *et al*. (2008) suggested that pH less than 5.0 and slightly higher than 7.4 produced a lethal effect on mosquito species. In most areas, it has been observed that only about a third to two thirds of all available habitats usually have anopheline larvae and only a few of these habitats produce a high number of adult vectors (Minakawa *et al*, 1999; Robert *et al*, 1998; Mutuku *et al*, 2006; Majambere *et al*, 2008). Significant association was observed between the young larvae of *An. gambiae* s. s. and *An. arabiensis* and the following physicochemical parameters of their breeding sites: dissolved oxygen (D.O.), nitrate (NO_3), total alkalinity, turbidity, and water surface temperature (Edillo *et al.,* 2006). Larvae of several mosquito species have been reported to use dissolved oxygen (DO) in addition to atmospheric oxygen (Clements 1992). However, as long as atmospheric oxygen is readily accessible, mosquito larvae are generally not affected by reduced Dissolved Oxygen (Dale *et al*. 2007). According to Ndenga *et al* (2012), habitats with high anopheline presence had greater abundance of mosquito aquatic stages and two times more levels of nitrate in water, whereas habitats with low anopheline presence had wider biofilm cover and higher levels of iron.

Heavy metals such as iron, lead, and arsenics are environmentally dangerous substances. Heavy metal pollutants, irrespective of the source, ultimately end up in aquatic systems. Low concentrations of heavy metals occur in natural aquatic ecosystems, but recent expansions in human population growth, industry, and peri-urban agricultural activities in African cities have led to an increase in heavy metal occurrence in excess of natural loads (Biney *et al*., 1994). Tiimub *et al.,* (2012) reported iron concentration of 0.12±0.01mg/l to 13.42±0.01mg/l and lead concentration of 0.01±0.01mg/l to 0.30±0.01mg/l in mosquito breeding habitat. This he said is much lower when compared with the Environmental Protection Agency (EPA) Maximum Permissible Limits. Heavy metals are more concentrated in man-made than in natural larval habitats (Mireji *et al*., 2008). Lead was associated with the presence of *An. gambiae* larvae in urban Kisumu. Absence of significant correlation between some of the metals and mosquito

species despite relatively high concentrations, suggest that the local larval populations, including key malaria vectors have adapted to the detected levels of these metals (Mireji *et al*., 2008).

2.2.3 Biological factors influencing *Anopheles* mosquito survival in their breeding habitats

Mosquito population dynamics are equally dependent on a number of biotic factors such as predation by carnivorous fish, competition for resources with other mosquito species in the habitat, aquatic plant species, hydrophytes, host choice and feeding preferences. *An. gambiae* s. s. and *An. funestus*, are known to breed in open sun-lit pools of water and relatively large permanent water bodies with vegetation, respectively (Gillies and Coetzee, 1987). *Anopheles* species require certain resources (food and shelter) for their survival and development (Adeleke *et al*., 2008). Availability of food nutrients for the larval stage of mosquitoes has been shown to affect the larval development (Scriber and Slansky, 1981). Most mosquito larvae are omnivores, feeding on living and non-living materials such as algae, fungi, bacteria, small metazoans, dust and insect scales. Interestingly, bacteria are the most important microbial constituents of mosquito larvae food and mosquito can grow on culture made only of bacteria (Merritt *et al*., 1992). In the environment, bacteria are widely distributed in *Anopheles* species breeding habitats and are used as food by mosquito larvae. Anopheline species such as *An. quadriannulatus* develop in relatively clear water by exploiting bacteria rich surface environment of permanent marshes through their interfacial feeding strategy (Yemane *et al*., 2000). Bacterial population such as *Bacillus* species, *Pseudomonas* species, *Micrococcus* species and *Serratia* species have been isolated in *Anopheles* mosquito breeding sites and were attributed to be a source of nutrient for the larvae (Chukalo and Abate, 2017). Therefore, the size of adult mosquito population is largely dependent on the nutritional role of organic matter and microbial fauna in the mosquito larval habitats (Okech *et al*., 2007).

Presence of detritus in *Anopheles* mosquito breeding sites equally ensures nutrient availability through their degradation ability. The longer the detritus is present in the larval habitat, the more microbial degradation which in turn might allow an increase in nutrient absorption by the larvae (Cummins and Klug, 1979). Lack of microorganisms was given as a reason for the failure of *An.*

albimanus to survive in the laboratory when fed exclusively on plant materials (Timmermann and Briegel, 1996).

The nutrient value of the breeding sites provides favourable conditions for the growth of bacteria, algae, fungal spores and protozoa which constitute the majority of food the mosquito larvae ingest. Gimnig *et al*. (2001) reported that *An. gambiae* s. l. was associated with turbid water, algae and the absence of emergent vegetation in small habitats. Gimnig *et al*. (2002) found that adequate food such as algae; bacterial composition and nitrogen were important regulators of *An. gambiae* larval growth. In the absence of predators the highest survival of larva has been observed at 66% algal cover. However, when the algae present in the habitat is of a greater quantity they pose a physical barrier to respiration and feeding activity of the larva thereby altering negatively the survival capability of the larva. Likewise a low quantity of algae reduces larvae survival by exposing them to a harmful level of ultra violet light.

On the contrary, some microorganisms cause reduction in the abundance of mosquito larval population. Two insecticidal bacteria have been used as larvicides to control larvae of nuisance and vector mosquitoes in many countries, *Bacillus thuringienesis israeliensis* and *B. sphaericus* (Wirth *et al*., 2010). Entomopathogenic fungi are unique as mosquito control agents because fungi have the ability to directly infect the host insect by penetrating into the cuticle and do not need to be ingested by the insect to cause disease. Extracellular secondary metabolites from many fungi have been screened for larvicidal activity against mosquitoes. The secondary metabolites of entomopathogenic fungi *Chrysosporium* species (Priyanka *et al*., 2001; Priyanka and Prakash, 2003; Verma and Prakash, 2010; Soni and Prakash, 2010), *Fusarium* species (Prakash *et al*., 2010) have been screened as a potential larvicides successfully. *Chrysosporium tropicum* metabolites are effective against mixed population of adult mosquito (*Culex quinquefasciatus*, *An. stephensi* and *Aedes aegypti*) after purification with flash chromatography (Verma and Prakash, 2010). The metabolites of *Aspergillus niger* also has potentials as a biocontrol agents for *Cx. quinquefasciatus, An. stephensi* and other mosquitoes population in tropical countries where these vectors are significant (Soni and Prakash, 2011). *Beauveria*

bassiana and *Metarhizium anisopliae* caused high mortality of *An. gambiae* and *An. stephensi* larvae (Bukhari *et al*., 2010).

2.3 Biting and resting behavior of *Anopheles* mosquitoes

Based on High-Resolution Melting (HRM) analysis of vertebrate cytochrome b, *16S* rRNA and *COI* (Cytochrome c Oxidase sub unit 1 gene) PCR products, humans are the prominent blood-meal hosts of malaria vectors, even though very few of them bite non-human vertebrate hosts (Ogola *et al*., 2017). Generally, the biting rate is highest shortly after the mosquito density peaks, near breeding sites where adult mosquitoes emerge and around the edges of areas where humans are aggregated (Smith *et al*., 2005). Nevertheless, mosquitoes frequently visit human homes where they bite. Malaria mosquitoes have preferential feeding habits as some tend to favour feeding indoors, such as the African vectors *An*. *gambiae* s. s. and *An*. *funestus* s. s. (Costantini *et al*., 1999; Coetzee and Fontenille*,* 2004) and the Asian vector *An*. *stephensi* s. s. (Manouchehri *et al.,* 1976), while others such as *An*. *arabiensis* exhibit ambivalent feeding behaviour (Service, 1970). *An. arabiensis* often commence feeding early at night (Braack *et al*., 1994, Githeko *et al*., 1996; Taye *et al*., 2006., Tirados *et al*., 2006; Fornadel *et al*., 2010., Yohannes and Boelee, 2012) when most people are socializing outdoors (Tirados *et al*., 2006) and readily feeds both indoors and outdoors (Gillies and Coetzee, 1987; Geissbűhler *et al*., 2007). Other studies reporting an apparent shift in vector biting behaviour in response to vector control have concluded that most transmission still takes place indoors, because that is where most people are at night (Seyoum *et al.,* 2012; Huho *et al*., 2013). However, Sinka *et al.* (2010) collated findings from studies in recent decades, and showed that *An. gambiae* s. l. in fact bites almost as much outdoors as indoors.

Studies have shown that *An. arabiensis* in many locations will commence feeding outdoors in very early evening (Braack *et al*., 1994, Githeko *et al*., 1996, Tirados *et al*., 2006; Yohannes and Boelee, 2012) with varied peak biting period. Peak biting by *Anopheles gambiae* and *An. funestus* was reported between 23.00 and 05.00 hours, a period when most people are in bed and under nets if they have them (Maxwell *et al*., 1998). The fact that most of the bites from *An. gambiae* s. l. and *An. funestus* occur during hours of the night when most people are in bed was

the source of the enthusiasm for the use of insecticide-treated bed nets for malaria control in Africa. In some countries or geographic sub-regions however, these species have a biting peak well before midnight (Tirados *et al*., 2006, Geissbűhler *et al.,* 2007; Yohannes and Boelee, 2012), but elsewhere is most intense in the middle hours of the night (Taye *et al*., 2006; Fornadel *et al*., 2010) or in the very early hours of the morning near dawn (Braack *et al*., 1994, Githeko *et al*., 1996). *An. gambiae* and *An. funestus* tend to commence feeding later at night (Pates and Curtis, 2005, Sinka *et al.,* 2010) when most people have turned indoors. Most studies reported *An. gambiae* to have biting peaks somewhere between late at night to the early hours of the morning (Githeko *et al*., 1996; Charlwood *et al.,* 2003; Pates and Curtis, 2005; Geissbűhler *et al.,* 2007; Sinka *et al.,* 2010), although it may vary and even peak during the first half of the night in some locations (Charlwood *et al.,* 2003). A similar pattern of biting occurs in *An. funestus*, with a biting peak mostly reported between midnight and early morning (Githeko *et al*., 1996, Paine *et al.,* 1999; Pates and Curtis, 2005; Sinka *et al.,* 2010). The generalized situation outlined above appears to have become more fluid as insecticide pressure from IRS and ITN is selecting for populations which are increasingly outdoor feeding (Lindblade *et al.,* 2006; Reddy *et al.,* 2011, Russell *et al.,* 2011, Kitau *et al*., 2012) and to a time when people are available outdoors (Charlwood *et al.,* 2003; Sougoufara *et al.,* 2014).

Charlwood and Graves (1987) found a marked shift toward earlier biting by *An. farauti* when nets were introduced in Papua New Guinea. They attributed this not to a genetic change in the population but to the fact that mosquitoes returning to a netted village from egg laying during the night would have difficulty in obtaining a blood meal before dawn. Thus many would remain hungry during the day and would attempt to find a meal as soon as dusk falls. For *An. gambiae* s.l., two studies comparing treated and untreated villages have reported little or no difference in biting rhythm (Magesa *et al*., 1991, Qui˜nones *et al*., 1998), but two other studies (Njau *et al*., 1993; Mbogo *et al*., 1996) reported a marked shift in biting time in those houses. The above-mentioned studies were carried out soon after net introduction, when selection for genetic changes in behavior could hardly be expected. The average number of bites received per person per night was estimated to be 3.51 in total, of which 0.69 (19.7%) would occur outdoors (Bradley *et al*., 2015). In Uganda, outdoor biting densities of *An. gambiae* s. l. exceeded the indoor biting

densities throughout the night; while the indoor and outdoor human biting densities of *An. funestus* group were apparently equal (Kabbale *et al*., 2013). Three of the main vectors of malaria in Africa (*An. arabiensis, An. gambiae* s. s. and *An. funestus*) all have a preference for feeding close to ground level, which is manifested as a strong propensity of 77.3% – 100% for biting on lower leg, ankles and feet of people seated either indoors or outdoors, but somewhat randomly along the lower edge of the body in contact with the surface when lying down (Braack *et al*., 2015). The evidence from this present study also suggests that the preference for feeding especially at the ankle region of seated people is not necessarily targeted at ankles or feet *per se*, but is related to height above ground (Braack *et al*., 2015). This behaviour changes when people lie down, in which situation biting becomes more random along a band of the body (excluding head) in contact with the ground or resting substrate.

Most female *Anopheles* species are endophilic whereby they prefer to rest indoors during the period between the end of feeding and the onset of search for an oviposition site. Water, vegetation and amount of shade are important, as whether the species enters dwelling and rests there after feeding and whether the species flies some distance from breeding areas. Naturally endophilic species include *An*. g*ambiae s*. *s*. and *An*. *funestus* in Africa, *An*. *culicifacies* in India, and *An*. *minimus* in East and Southeast Asia (Pates and Curtis, 2005). Along Badagry axis of coastal lagoon in Nigeria, both species of *An. gambiae* s. s. and *An. melas* were found to be endophilic and endophagic in contrast to *An. nili* with low indoor density (Oyewole *et al*., 2010). Despite being endophilic, *An*. *darlingi* appears to have developed a shorter indoor resting period in Suriname and Colombia, owing to insecticide pressure (Rozendaal *et al*., 1989). In Greece in the 1950s, DDT spraying programmes led to exophilic behaviour in the originally endophilic *An. sacharovi* which possibly became more zoophilic during the spraying campaign (Boreham and Garrett-Jones, 1973). Similarly, malaria transmission by *An. sundaicus* in southern Java was not controlled by DDT house spraying, because of increased exophily (Sundavaraman, 1958). A decrease in the number of *An*. *sundaicus* resting on sprayed walls at night was observed, yet large numbers were still recovered from human bait. This “bite and run” behaviour has been shown in populations of *An. gambiae* s. s in the Tanga region of Tanzania (Gerold, 1977), where high proportions leaving the sprayed house were fully engorged and displayed a high flight

activity. Similarly, a 94% exit rate of *An. gambiae* s. s and *An. funestus* from pyrethroid-treated huts was observed in Burkina Faso (Darriet, 1991). In Tanzania, Mnzava *et al*. (1995) found that a higher number of fed *An. arabiensis* were exiting DDT-sprayed houses than in lambdacyhalothrin sprayed houses, from which most exiting mosquitoes were unfed. Lambdacyhalothrin treated area, had a higher impact on malaria transmission than did DDT because it acted rapidly by either deterring mosquitoes from feeding or killing them. In India, naturally endophilic *An. fluviatilis* were found resting largely outdoors and only entering houses to take blood; there is also the existence of outdoor resting populations of the major malaria vector, *An. culicifacies* (Sharma, 2003). Entomological surveys have shown a complete change in the behaviour of *An. minimus* in Hainan Island, China, which is now endophagic and exophilic, and has an equal preference for humans and cattle (Li *et al*., 1983). *An. dirus* is a known endophagic but exophilic mosquito in Hainai Province in China (Curtis *et al.*, 1990). In a recent study, Müller *et al* (2017) reported a higher house exit rate of *An. gambiae* s. s.

2.4 Entomological indices of malaria transmission

2.4.1 Blood meal sources of *Anopheles* mosquitoes

Adult female mosquitoes take blood meal to obtain nutrients needed for egg development. Thus blood meal analysis is a very important epidemiological factor in malaria studies. It could show the feeding preference of *Anopheles* mosquitoes collected in a given locale. Identifying *Anopheles* mosquito blood meal source helps in determining the degree of contact between the malaria vectors and host populations. In analyses, the host range of a given *Anopheles* species is crucial to assessing risk of human exposure to malaria parasites and changes in *Anopheles* trophic preference (Githeko *et al*., 1994). Okwa *et al* (2009) reported that *Anopheles* species collected in the rain forest zone of Nigeria had more bovine than human blood whereas 73.3% of collections in the savannah area fed on human. The human blood index reported was 57.3%. Human IgG was detected in 98.97% of *An. gambiae* s. l. and 99.48% of *An. funestus* collected along the Kenyan coast; with the remaining feeding on other vertebrate hosts tested, which were bovines, chickens, and goats (Mwangangi *et al*., 2003). With the availability of cattle and other domestic animals, high proportion of *An. gambiae* s. l. and *An. funestus* were reported to have fed on human (Mbogo *et al*., 1993). This is unlike the situation in western Kenya (Githeko *et al*.,

1994) and in the Mwea irrigation scheme (Ijumba *et al*., 1990), where the availability of cows is a determining factor for blood feeding. In a more recent study, Ndenga *et al*. (2016) reported that 53.1 % of *An. gambiae* s. s. obtained blood-meals from humans, 26.5 % from goats and 18.4 % from bovines. In Papua New Guinea, 52.9% of *An. punctulatus* s. l. collected in a study fed on human, 15.8% on dog and 29.2% on pigs; there was also detection of DNA from mice, one marsupial species and two bat species (Logue *et al*., 2016).

In areas where bed net coverage is high, malaria vectors might find it increasingly difficult to find a successful blood meal from their favourite host (Magesa *et al*., 1991; Gimnig *et al*., 2003). There is a possibility that the malaria vectors obtain blood meals from all available domesticated animals, as shown by a large number of previous and recent studies indicating that the major malaria vectors in sub-Saharan Africa readily adapt to available blood-meal hosts even if they have a preference for human hosts (Githeko *et al*., 1994; Mwangangi *et al*., 2003; Okwa *et al*., 2011; Animut *et al*., 2013; Massebo *et al*., 2013; Ngom *et al*., 2013). Low Human Blood Index (HBI) of 53% in the work of Ndenga *et al*. (2016) strongly suggests a shift in blood meal sources as a result of the interaction between increased bed net coverage and close proximity of domesticated animals. He reported single blood meal sources from human, bovine and goat as well as mixed blood meal sources by *An. gambiae* s. s. Mixed blood meal source was also reported in *An. punctulatus s. l.* with 16.3% of the mosquitoes feeding on more than one host species and 4.9% of the mosquitoes unambiguously feeding on more than one person (Logue *et al*., 2016).

2.4.2 Sporozoite rates of *Anopheles* mosquitoes

The transmission of malaria parasites requires the survival and development of *Plasmodium* species within two individual organisms: the vertebrate host and the mosquito vector. Thus measuring transmission from human populations to mosquitoes in natural settings is an important complement to measuring Entomological Inoculation Rate (EIR). Transmission of parasites from humans to mosquitoes involves uptake of at least one mature male gametocyte and one mature female gametocyte in a blood meal. As a result, gametocyte density in the host blood is a determinant of the infection success in the mosquito. The number of gametocytes ingested by the

mosquito from the vertebrate host has been identified as one of the main variables that influence the success of *Plasmodium* infection in the vector.

The basic understanding of malaria transmission requires measuring the probability that a mosquito would become infected after feeding on a human. Of the approximately 400 *Anopheles* species, some are more suitable hosts for *Plasmodium* species than are others. A nulliparous mosquito cannot transmit malaria because it has not obtained the *Plasmodium* parasite yet. Even for a female that has laid eggs once (or twice), it may not yet be old enough to transmit malaria parasites because the gonotrophic cycle – the time from the first blood seeking to the second blood seeking averages only three days and sporozoite development takes 10-12 days. Therefore, a mosquito may need three gonotrophic cycles before it is able to transmit malaria parasites (WHO, 2003). After a female mosquito takes a blood meal, microgametocytes and macrogametocytes are transformed into the respective gametes within the mosquito's midgut. The gametes undergo fusion to form a zygote, which in turn transforms into an elongated motile form called ookinete within 18hrs - 24hrs. Malarial ookinete traverse single epithelial cell or additionally some neighboring epithelial cells before they arrive at the basal lamina; indicating that cell traversal ability is essential for the ookinete to establish infection in the mosquito vector (Kariu *et al.*, 2006). A recently proposed model of ookinete infection of the midgut (Zieler and Dvorak, 2000; Han and Baullas-Mury, 2002) suggests that epithelial cells invaded by ookinete soon die and then ejected from midgut wall, which exposes the parasite to danger of being removed from the epithelium with the damaged cells and requires the ookinetes to cross this layer in a limited time. Thus prompt passage through the epithelial cell is critical for the parasite successful infection of the mosquito vector. Also during this midgut invasion, many ookinetes are killed by the insect defence system and the number of malaria parasite is greatly reduced (Blandin and Levashina, 2004). When ookinete penetrates the wall of the midgut, it becomes a round oocyst which is sessile. Inside the oocyst, the nucleus divides repeatedly, with the formation of a large number of sporozoites and the enlargement of the oocyst. When the sporozoites are fully formed, the oocyst bursts, releasing the sporozoites into the mosquito's body cavity. The sporozoites then migrate to the salivary gland of the mosquito for transmission into another host. Infection of human host with *Plasmodium* parasites begins with the bite of an

infected female *Anopheles* mosquito that can inoculate the individual with sporozoites. In general, it is believed that only approximately 10-100 sporozoites are inoculated during a blood meal, and an even smaller number actually enter the circulation (Hall and Fauci, 2009).To become infectious, an infected mosquito must survive long enough for the oocyst to rupture and releases sporozoites which must migrate through the mosquito haemocoel to reach the salivary glands; this process takes several days (Vaughan, 2007).

Currently it is assumed that all mosquitoes with salivary gland sporozoites are equally infectious irrespective of the number of parasites they harbour, though this has never been rigorously tested. However, mosquitoes with a higher numbers of sporozoites in their salivary glands following blood-feeding are more likely to have caused infection (and have done so quicker) than mosquitoes with fewer parasites (Churcher *et al*., 2017). Here the probability of infection may also increase with the number of infectious bites. It appears intuitive that transmission will increase with the size of the inoculums though there is little empirical evidence to support this assumption and no direct evidence from human malaria. The work of Churcher *et al*. (2017) also provides direct evidence to suggest that the world's first licensed malaria vaccine may be partially effective because it fails to provide protection against highly infected mosquitoes. However, many highly infected mosquitoes may fail to inject sporozoites during blood feeding (Frischknecht *et al*., 2004; Medica and Sinnis, 2005; Jin *et al*., 2007)

Among *Anopheles* species, there are tendencies for significantly higher parity or sporozoite rates among those that bite in the earlier hours of the day. The proportion of the sporozoite positive night bites by *An. gambiae s. s.* and *An. funestus* occurring between 2200 and 0500h was about 88% (Maxwell *et al*., 1998). In a study in Kenya where 416 engorged *Anopheles* mosquitoes were analysed for *P. falciparum* infection by high-resolution melting (HRM) analysis of 18S rRNA and cytochrome b PCR products, 41 tested positive (Ogola *et al*., 2017). The *P. falciparum* infection rates were 10.00%, 11.76%, 0%, and 18.75% among blood-fed *An. gambiae* s. s., *An. arabiensis*, *An. funestus* s. s. and *An. coustani* respectively. In Nigeria Oduola *et al*. (2012), working in Oyo, south-western Nigeria, found that *P. falciparum* sporozoite infection rate of *An. gambiae* s. s. varied between 1.9% and 3.1% in the study communities. Earlier, low

sporozoite rates of 7.6% and 1.4% had been reported in *An. gambiae s. s.* and *An. arabiensis* respectively during the Garki Malaria Control Programme (Molineaux and Gramiccia, 1980). However, high sporozoite rates of 31.5% and 17.9% were reported for *An. gambiae* s. l. and *An. funestus* respectively in Makurdi Nigeria (Msugh-Ter *et al*., 2014). In Makurdi, only *An. gambiae s. l.* and *An. funestus* were found to be the major malaria vector groups involved in malaria transmission in the study area as they were found to be significantly infected in this area (Msugh-Ter *et al*., 2014)

Main vectors of malaria parasite, *An. gambiae* s. s and *An. melas* collected along Badagry axis of coastal lagoon in Nigeria had an overall *P. falciparum* sporozoite infection rate of 4.8% and 6.5% respectively; with all the *An. nili* testing negative for *P. falciparum* sporozoite infection (Oyewole *et al.*, 2010). In another study, *An. gambiae* had an infection rate of 12.6 % across collection sites (Sanford *et al*., 2014). The sporozoite infection rates in all the four localities in the study area for all the *Anopheles* species were higher in the wet season months as compared to the dry season periods (Msugh-Ter *et al.,* 2014). This is completely in agreement with the findings of Wanji *et al.* (2003) who found higher sporozoite rates for both *An. gambiae* and *An. funestus* in the wet season than in the dry season in the mount of Cameroon region.

Factors such as temperature, humidity and rainfall directly impact the lifecycle of malaria parasites. Higher temperatures accelerate *Plasmodium* species growth within *Anopheles* mosquitoes (CDC, 2012). Temperature between 15^0C - 40^0C and humidity between 55% and 80% are suitable for the completion of the *P. falciparum* and *P. vivax* malaria parasites life cycle (Zhou *et al*., 2004).

2.4.3 Entomological Innoculation Rate (EIR) of *Anopheles* mosquitoes.

Entomological Innoculation Rate measures the intensity of malaria parasite transmission by *Anopheles* species. It is a direct measurement for assessing the impact of vector control tools on malaria transmission. It is the number of infective bite per person per night and is calculated as the product of the human biting rate and the sporozoite rate (WHO, 2003). EIR of 5.3 infectious bites per person (ib/p) per eight months was reported for *An. arabiensis* in Southwest Ethiopia

(Abraham *et al*., 2017). The EIR's were 0, 61.79 and 6.91 bites/person/night for Fort Ternan, Lunyerere and Nyalenda villages in Kenya (Imbahale *et al*., 2012). Very high EIRs of 23, 53 and 61 were recorded in three different areas of Gabon (Sylla *et al*., 2000). However in Dienga and Benguia areas of Gabon, mean EIR of 0.28 infective bites/person/night and 0.76 infective bite/person/night respectively were reported (Elissa *et al*., 2003)

A study, in Dar es Salaam, measured EIR differences to assess the impact of source reduction in malaria control. The control area had an annual EIR of 1.06 and the area that received the microbial larvicide *Bacillus thuringiensis israeliensis* (*Bti*) had an annual EIR of 0.56 (Geissbuhler *et al*., 2009). There was a lower EIR reduction of 47% due to source reduction compared to the ITN and IRS studies (Geissbuhler *et al*., 2009). However, source reduction is likely to be particularly effective in urban areas, where breeding places are man-made and can be identified, mapped, and treated (Gu *et al*., 2006).

2.5 Epidemiology of malaria

2.5.1 Malaria parasite prevalence

Malaria is one of the most prevalent human infections worldwide. It is still a major public health challenge in most of the sub-Saharan African countries. In 2015, of the estimated 214 million cases of malaria, 88% were diagnosed in this region alone (WHO, 2016) with the highest burden shared by pregnant women and children under 5 years. In Nigeria, malaria is the number one public health problem (Onwujekwe *et al.*, 2000; FMH, 2001) and has been responsible for about 300,000 deaths every year (Coker *et al.*, 2001; WHO, 2003). In Africa, malaria had been reported to be responsible for about 20-30% hospital admissions and about 30-50% of outpatient consultations (UNICEF, 2000). Approximately 50% of the Nigerian population experience at least one episode per year. However, official estimate suggested as much as four bouts per person per year on the average (WHO, 2003). Malaria accounts for 40% public health expenditure (USAID Health, 2005). The cost of malaria treatment and prevention in Nigeria had been estimated to be over $1 billion per annum (Odaibo, 2006).

In Nigeria, malaria prevalence in children between 6 to 59 months based on geographical coverage was 41-50% in the North-West, North-Central and South-West; 31-40% in North-East and South-South; and 21-30% in the South-East (Nigeria Malaria Fact Sheet, 2011). Several other studies have reported the prevalence of malaria among school age children in several locations in Nigeria (Okafor and Oko-Ose, 2012; Umaru and Uyaiabasi, 2015; Nmadu *et al*., 2015; Abah and Temple, 2015; Okeke *et al*., 2016; Bassey and Nwakaku, 2017). Also, there are several deaths emanating from malaria and its co-infections. For instance, Nigeria Malaria Fact Sheet (2011) reported that 100 million malaria cases occurred in Nigeria leading to about 300,000 deaths per annum, making malaria the leading cause of death after HIV/AIDS in Africa.

2.5.2 Malaria endemicity

Malaria endemicity is a measure of malaria parasite prevalence in a particular region; where prevalence is the proportion of people infected at a given point in time. Malaria risk is often stratified into three broad categories depending on the degree of transmission. They are malaria free areas, unstable or epidemic areas and stable or endemic areas (WHO, 2003). In a case of stable malaria transmission, the adult population usually shows a high level of immunity to malaria and therefore children are more often at risk of severe disease and death due to malaria than are adults. Area of unstable transmission is prone to epidemics. Some of the characteristics of such areas include: high seasonal transmission, climatic conditions favourable for short periods of transmission and high anopheline density required to sustain transmission.

The level of endemicity within an area is of great interest as it provides information about areas of stable and unstable malaria transmission. Spleen rate and parasite rate among population are used in measuring endemicity levels (WHO, 2003). The number of palpable enlarged spleen or persons with parasitaemia is calculated. Areas presenting the same level of endemicity are likely to have similar characteristics of disease distribution. In hypoendemic areas, there is intermittent, very little transmission and low risk of infection to the population. The parasite rate here is usually less than 10%. In mesoendemic areas, there is varying seasonal transmission with low intensity of malaria. The affected area is often prone to malaria epidemics. The parasite rate here is usually from 11% to 50%. In hyperendemic areas, there is intense seasonal transmission of

malaria. Periods of no transmission occur during the dry season. The parasite rate is from 51% to 75% among the population. However, the transmission rate is not sufficient enough for a very high proportion of the population to develop protective immunity. In other words, the area of transmission can still be described as unstable. In holoendemic areas, malaria parasite transmission occurs all year round. There is a high degree of immunity among the population of all age groups, particularly adults. The parasite rate here is usually greater than 75%. There is stable transmission with anaemia being most severe in early life.

2.5.3 Factors influencing malaria prevalence, intensity and endemicity

The risk of malaria infection and morbidity is difficult to estimate accurately due to the interplay of the epidemiological parameters. Even within a single country, there are considerable variations in malaria epidemiology due to differences in parasite, human, vector and ecological factors (WHO, 2005).

2.5.3.1 Factors relating to *Plasmodium* species.

Malaria infection is mostly acquired in areas where human hosts carrying the *Plasmodium* parasites are found in addition to presence of enough Anopheline mosquitoes under suitable environmental conditions (Ani, 2004). *P. falciparum* is the most commonly encountered species in West Africa including Nigeria (Mbanugo and Ejims, 2000). *P. malariae* has a much lower prevalence than *P. falciparum* and *P. vivax.* It accounts for up to a third of *Plasmodium* infection in tropical Africa (Miller *et al.*, 1976). It was found to contribute to 13.6% of malaria in Uli, Anambra State of Nigeria (Onyido *et al.,* 2011). *P. falciparum* was responsible for 40.08% malaria prevalence among primary school children in Ebonyi State (Ani, 2004). It is also found in India, Guyana, Malaysia and Sri Lanka. In these countries, it accounts for less than 10% of *Plasmodium* infections. Although *P. vivax* malaria is found throughout Asia, South and Central America, Oceania, Middle East, it also occurs in some parts of Africa. It is the second most virulent malaria species after *P. falciparum* and has put nearly 2.85 billion people at risk of infection (WHO, 2013)**.** *P. ovale* is of low prevalence and has a restricted distribution. It is found

mainly in West Africa where it accounts for up to 10% of malaria infections. In Anambra State of Nigeria, Onyido *et al.* (2011) reported 4.4% prevalence of *P. ovale*.

2.5.3.2 Factors relating to humans

Prevalence of malaria could be affected by life style of a given locality, population densities, and cultural practices among others (Umaru and Uyaiabasi, 2015). Nigeria has a wide range of cultures. This is one of the main determinants affecting the transmission, intensity and management of malaria infection (Umaru and Uyaiabasi, 2015). These practices and activities of humans enhance human-vector contact and as such affect the prevalence of malaria parasites. Some of the associated attributes and attitudes include demography, environmental sanitation practices and drainage pattern (Ukpong *et al*., 2015). The breeding of mosquito could be traced to poor sanitation which may contribute to high prevalence and intensity of malaria. This is because poor sanitation such as dirty and non-flowing drainage systems, indiscriminate dumping of wastes without appropriate disposal mechanisms provides breeding grounds for malaria vectors (Pam *et al*., 2015). Land use such as deforestation and swamp reclamation by eliminating shade modifies the local climate and microclimate, and in the presence of stagnant water, new habitats for malaria vectors are formed (Munga *et al*., 2006, Lindblede *et al*., 2000). Consequently, the new habitats provide new breeding grounds leading to increased vector densities and subsequently an increase in malaria transmission.

Malaria is no respecter of persons, so all groups of people are exposed to the infectious bites of the female *Anopheles* mosquito, which breeds in stagnant water (Hay *et al*, 2000). However, Malaria disproportionately affects the poor, in whom higher morbidity and mortality can largely be attributed to inadequate protection and lack of access to effective treatment. Asymptomatic individuals are able to sustain malaria transmission.

Mobility of human hosts between different regions influence the transmission process (Yadav *et al*, 2005; Singh *et al.*, 2004). Through movement, people are exposed to malaria risks in a variety of ways which include exposure of people with low or no immunity to infected persons

(Prothero, 1977). The daily or weekly circulation of hunters, gatherers and farmers may involve movements between areas which differ ecologically in minor respects but still increase contacts with vector breeding habitats (Singhanetra-Renard, 1993). The most pathogenic human malaria species, *P. falciparum* most likely originated in Africa probably in the past 5,000-10,000 years with the onset of Agriculture (Coluzzi, 1999). Forest clearing either for farming or other purposes has allowed populations to enter areas that malaria had previously rendered uninhabitable. As a result, there is destruction of animal habitats, and a decline in the number of wild animals which forces the mosquitoes to feed on domestic animals and humans (Walsh *et al*, 1993). Agricultural changes have created numerous water bodies exposed to the sun, providing ideal conditions for vector proliferation and increased malaria transmission (Lindblade *et al*., 2000; Briet *et al*., 2003). The development of African agriculture in the forest clearing resulted in the vectors most important characteristics of malaria transmission whereby the mosquitoes almost exclusively bite human (Coluzzi, 1999).

Influx of people into an area can also increase the spread of malaria. As urban centres continue to grow, the scale and impact of urban malaria is increasing (Breman *et al,* 2004). The consequences of urbanization suggest that unplanned urban growth alters the frequency and transmission dynamics of malaria (Keiser *et al*, 2004). Most sub-Saharan African countries are experiencing unprecedented rate of population growth which has led to uncontrolled and unsustainable exploitation of natural resources, especially the forests resources. Through the process of forest clearing, deforestation alters every element of local ecosystems such as microclimate, soil, and aquatic conditions, and most significantly, the ecology of local flora and fauna, including malaria vectors (Uneke, 2009). Importantly, the declining economies of the African countries and their cities struggling to cope with the pace and the extent of urbanization, poses unique challenges to the control of the diseases as poor housing, lack of sanitation and drainage water surface increases vector breeding and human vector contact (Martens and Hall, 2000).

2.5.3.3 Factors relating to *Anopheles* species

The distribution and abundance of *Anopheles* species are the key determinants of malaria parasite transmission. The differences in malaria risk among the sites can be explained by vector species of local importance and availability of breeding habitats (Imbahale *et al*., 2011). A strong positive correlation has been observed between malaria prevalence and abundance of *Anopheles* mosquitoes in Ogbunike, Anambra state (Onyido *et al.*, 2011).

Existence of *Anopheles* species and their complexes, their varied behavior and involvement in malaria transmission in the different ecological settings are some of the factors that affect malaria prevalence and endemicity (Kouznetsov *et al*., 1986). Malaria in sub-Saharan Africa is transmitted by a range of *Anopheles* mosquitoes and the risk of infection and disease vary greatly across the continent. *Anopheles* species complexes containing morphologically cryptic sibling or isomorphic forms presents a major challenge to malaria control programmes as these require vector identification using molecular techniques (Kouznetsov *et al*., 1986). *An. gambiae* s. l. Giles (Diptera: Culicidae) is one of the most important malaria vectors in Africa, where 90% of the world malaria cases occur (Chandre *et al*., 1999). The complex consists of at least seven sibling species, five of which are vectors of human malaria parasites with varying degree of efficiencies (Coluzzi *et al*., 1984; Gillies and Coetzee, 1987; Hunt *et al*., 1998). The combination of anthropophily and endophily puts both *An. gambiae* and *An. funestus* in a special place in malaria study. This is corroborated by the findings of Coetzee (2000) who reported that high survival rate coupled with anthropophily and endophily, ensure that across Africa, both species are responsible for most of the malaria transmission. *An. arabiensis*, which in turn exhibits greater ecological flexibility than other members of the *An. gambiae* complex from a historical perspective (Meyrowitsch *et al*., 2011) is also a major problem.

Because of the significance of *Anopheles* species in malaria transmission, malaria control has relied heavily on indoor vector management; primarily indoor residual spraying and use of insecticide treated bed nets. The main entomological justification for the use of ITNs was that most biting by the anthropophilic, endophagic and endophilic vector mosquitoes occurred at hours of the night when most people were in bed and under nets if they had them (Maxwell *et*

al., 1998). However, evidence of exophily by the major malaria vector *An. gambiae* raises concern about the long term effectiveness of Indoor Residual Spraying (IRS) and Long Lasting Insecticide-treated Nets (LLINs) in reducing outdoor transmission of malaria especially before bedtime and by people sleeping outdoors. In a study, malaria infection was not significantly higher in individuals who reported spending time outside between 7 pm and 6 am the previous night compared to those not spending time outside in both adults and children (Bradley *et al*., 2015). Since the 1950s, outdoor biting (exophagy) and outdoor resting (exophily) have been recognized as important factors reducing the effectiveness of IRS and its capacity to interrupt transmission (Pates and Curtis, 2005). It has been argued that because of exophagy and exophily, additional interventions may be necessary to maintain reductions in malaria transmission and to achieve malaria elimination (Govella and Ferguson, 2012; Pates and Curtis, 2005; Russell *et al*., 2013). The relatively high infection rate of 11.1% of *An. melas* in Guinea-Bissau together with its tendencies to be both endophilic and exophilic and having a high human blood index (Sharp *et al.*, 2007; Tuno *et al.*, 2010) make the species a significant vector, which may also be hard to control by reliance on ITNs and LLINs. While numerous entomological studies (Russell *et al*., 2011; Govella *et al*., 2010; Seyoum *et al.,* 2012) and mathematical models (Killeen and Chitnis, 2014; Govella *et al*., 2010) have investigated the impact of exophagy, little evidence however has been published linking outdoor biting to epidemiological malaria outcomes. Also, Various vector control methods and anti-malarial drugs are now available to decrease transmission and cure infections, but universal coverage with these interventions may be insufficient to interrupt transmission in areas where potential transmission intensity is very high (Gething *et al*., 2011; Smith *et al*., 2007).

Malaria mosquito abundance and biting rates vary markedly in space and time. Spatially, the variables can vary over the space of a few kilometres (Kulkarni *et al*., 2010; Mboera *et al*., 2010). This spatial heterogeneity in abundance and biting indicates variation in environmental conditions that affect mosquito distribution (Ernst *et al*., 2006; Kulkarni *et al*., 2010). It has been shown before that housing characteristics can affect malaria risk, and future developments in housing quality could have a large impact on malaria transmission on Bioko (Bradley *et al*., 2013). House design features that impede mosquito entry are expected to add to the protection of

people indoors, but it might also increase the relative importance of mosquitoes biting outdoors. Other effects may include changes in biting behaviour expressed by outdoor biting and/or time of biting (Mbogo, *et al*., 1996) as well as change in host preference as the favoured host is under the ITN (Takken, 2002). The main vectors of malaria in Dar es Salaam are *An. gambiae* s. s., *An. arabiensis*, *An. funestus* and *An. merus* (Geissbühler *et al*., 2007). Interestingly, these malaria vectors in Dar es Salaam appear to have adapted to high coverage with bed nets and improved housing by predominantly feeding outdoors (Geissbühler *et al*., 2007). Thus, insecticide-treated nets confer slightly less protection than in rural areas with poor houses; so additional measures directed at aquatic stages of vector mosquitoes may have a useful role in this and similar urban settings (Geissbühler *et al*., 2007).

Reasons for this are thought to include change in the biting pattern of a greater proportion of the malaria vectors to biting earlier or later in the night and biting outdoors when many people are not in bed, rendering bed nets less effective (Maxwell *et al.,* 1998), hence causing an increase in the malaria infection rates. Results of a study shows that people who wake up before dawn particularly those who wake up early for trade and farming activities in the urban/semi-urban centers and rural areas respectively are at risk of getting many mosquito bites as they get exposed to the later biting cycle of the vectors, with higher chances of contracting the infections they transmit, namely: *Plasmodia* and filariases (Jan and WHO, 1997). The results of some studies, however, contrast with results of other studies, for example, in another study in Kenya, biting occurred earlier in the evening following ITN use (Mbogo *et al*., 1996). In Papua New Guinea and Tanzania studies, shifts in time of biting were observed where mosquito biting occurred earlier in the evening as hosts had not yet gone to bed and were easily accessible (Takken, 2002). In a study to test bed net traps for monitoring mosquito populations and time of biting in Tanzania and possible impact of prolonged use of insecticide treated bet nets, it was observed that somewhat more of the *Anopheles* biting occurring early and late in villages with ITNs, whereas in villages with no history of ITN use, biting was concentrated in the middle of the night.

Transmission reduction is an integral part of efforts to control and eliminate malaria (Alonso *et al*., 2011). However, the resilience of *Anopheles* mosquito infection to changes in malaria endemicity has an implication in planning control. Gametocyte density in the host blood is a determinant of the infection success in the mosquito. In some areas where intervention has taken place, asexual parasite prevalence in humans has reduced from 70% to 20%, but that the proportion of infectious mosquitoes has remained roughly constant. Evidence suggests that this is due to an increase in transmission efficiency caused by a rise in gametocyte densities, although the uneven distribution of mosquito bites between hosts could also contribute (Churcher *et al*., 2014). More efficient transmission represents more mosquitoes being infected at a given endemicity. Humans are therefore exposed to mosquito bites, which are probably malaria sporozoite-positive, all year round as the *An. gambiae* and *An. funestus* species that are highly anthropophilic and endophilic (White, 1998, Echodu *et al*., 2010) are prevalent throughout the year particularly in Budiope County of Uganda. However, the proportion of infected mosquitoes may depend on the mosquito death rate (Smith and McKenzie, 2004). Also, spatio-temporal fluctuations in mosquito densities commonly alter the age-structure of mosquito populations. Owing to the time the parasite needs to develop, the older a mosquito, the more likely it is to be infected or infectious. Therefore, emergence of a large cohort of young adult mosquitoes, driven by seasonal rainfall for example, would instantly reduce the sporozoite rate in a mosquito population. Thus in measuring infection rates of mosquitoes, it becomes possible to infer the probability that a parasite is transmitted from a single infectious human to a mosquito and the proportion of mosquitoes in a population that become infected after biting a human. The ability of adult *Anopheles* mosquitoes to survive for at least 10 days makes malaria parasite transmission very possible.

2.5.3.4 Environmental and climatic factors

Nigeria has a wide range of micro-weather conditions which can affect the prevalence of malaria in a given locality (Umaru and Uyaiabasi, 2015). According to Okonko *et al*. (2009) environmental conditions that favour the breeding of mosquitoes enhances the proliferation of the *Plasmodium* species. Sanganuwan and Adelaiye (2007) reported high malaria incidence rate

of 78.25% in children under 9 years in University of Agriculture, Makurdi Health Centre and attributed this to environmental conditions of the area. Amuta *et al*. (2014) reported a similar high malaria infection rate of 68.3% in pregnant women in Makurdi.

Even in one topographic area, mosquito vectors and malaria infections may not be distributed homogeneously, and some households within the same area have a higher malaria incidence than others (Munyekenye *et al*., 2005; Brooker *et al*., 2004; Carter *et al*., 2000). Results of study by Imbahale *et al*. (2012) show a heterogeneous distribution of vectors and the risk of being infected with malaria within sites only a few kilometers apart. He stated that the differences in malaria risk among the sites can be explained by topography, terrain characteristic (Atieli *et al*., 2011), and environmental conditions among others.

In many areas where malaria transmission occurs, the epidemiological pattern is mostly unstable and seasonal, but its intensity differs from place to place due to altitudinal variation (Adhanom *et al*., 2006). The areas with altitude 2000m above sea level were considered as malaria-free, but currently areas above 2400m above sea level experience local malaria transmission (Woyessa *et al*., 2004; Graves *et al*., 2009). The expansion of malaria transmission to highland areas has been influenced by the change in climatic conditions (Siraj *et al*., 2014). Moreover, the population in areas with unstable malaria transmission is non-immune and can experiences malaria epidemics (Lindsay and Martens, 1998) including the highlands of Ethiopia (Woyessa *et al.,* 2012). Travelling from the highlands to the lowlands for daily activities increases the risk of malaria infection in highland residents (Alemum *et al.,* 2014). Therefore, the geographical expansion of malaria to the highlands could be a serious public health concern because most of the Ethiopian population lives in the highlands of the country.

The transmission dynamics of malaria is also influenced by a mixture of climatic factors which impact on the vectors ecology and also capable of enhancing transmission rates and patterns in certain areas (Ukpong *et al*., 2015). Environmental attributes including rainfall and its pattern, relative humidity and temperature are some of the determinants that affect the ecology of the

vector of malaria (Alaba and Alaba, 2009). According to Ukpong *et al*. (2015), temperature plays a significant role in determining the transmission dynamics of the vector as well as the parasite growth and development. Thus the development of the parasite within the mosquito (sporogonic cycle) is dependent on temperature. The authors further reported that extrinsic incubation period of malaria parasite within the vectors is significantly affected by temperature. Increasing temperature may shorten the developmental time of the malaria parasite within the mosquito vector and hasten the development of immature stages of *Anopheles* mosquitoes (Githeko and Lindsay, 2000; Minakawa *et al.,* 2006). It takes about 9 to 10 days at temperatures of 28°C, but stops at temperatures below 16°C. The minimum temperature for the development of *P. falciparum* and *P.vivax* within the vector is approximately 18°C and 15°C respectively (Craig *et al*, 1999). Especially, ambient temperature plays a major role in the life cycle of the malaria vector. The feeding rate and blood digestion frequency of the adult female *Anopheles* mosquitoes increase in warmer temperatures (Ukpong *et al*., 2015). Also the daily survival of the vector is dependent on temperature. At temperatures between 16°C and 36°C, the daily survival is about 90%. The highest proportion of vectors surviving the incubation period is observed at temperatures between 28°C - 32°C (Craig *et al*., 1999; Jonathan *et al*., 2006).

Malaria occurrence in tropical and sub tropical Africa is mostly seasonal with its major incidence occurring in the rainy season (Eneanya, 1998; Oesterholt *et al.*, 2006). Population density of the *Anopheles* species increased tremendously between May and June and this corresponds to the peak of rains. Also, peak of malaria during rainy season occurred in May (Okullo *et al*., 2017). Rainfall provides breeding sites for mosquitoes to lay their eggs, and ensures a suitable relative humidity of at least 50 to 60% to prolong mosquito survival (Reiter, 2001). During this period, higher sporozoite rates and Entomological Inoculation Rate (EIR) are recorded compared to the other months (Oyewole *et al*., 2010). Excessive rainfall during certain period was associated with increased malaria in south-western highlands of Uganda (Kilian *et al*, 1999). However, a study in Ethiopia had reported that although an epidemic was associated with higher rainfall (Woube, 1997), an epidemic in another year was preceded by very little rain. Similarly, a reduction in malaria infection occurred in the Usambara Mountains of Tanzania following 2.4 times more rainfall than normal (Lindsay *et al*, 2000). Another study found variation in the relationship

between the mosquito population and rainfall in different districts of Kenya and attributed it to variation in environmental heterogeneity (Mbogo *et al.*, 2003). *An. gambiae* generally increases in density after the start of the long rains, while *An. funestus* density is seen to vary in direct proportion to the proximity of permanent breeding grounds rather than rainfall (Garnham, 1929). Studies had indicated that rainfall seems to be a critical feature of habitat differences, especially between the molecular forms of *An. gambiae* s. s. (Edillo *et al*; 2002).

In Budiope county, both *An. gambiae s. l.* and *An. funestus* mosquitoes thrived all year round regardless of the amount of rainfall (Kabbale *et al*., 2013). In Uganda, higher catches of the *Anophele*s species were realized during the rainy seasons (March to May and July to October) or at onset of the rains , this being attributed to the fact that the rainy season provides water for breeding (Echodu *et al*., 2010) and increases humidity for mosquito survival (White, 1998). Densities of *An. gambiae s. l.* were observed to increase following the peak of rainfall (April and July 2010) in Uganda, while *An. funestus* densities increased at the end of the rainy season and at the beginning of the dry season. This finding could explain the all-year-round malaria transmission as the two groups of mosquito peak at different times and hence prolonging the transmission period through the year in Kamuli district and most parts of Uganda. Therefore, even during the dry periods of the year this swamp is continuously productive for *An. funestus* mosquitoes. Heavy rainfall over a short period may create new pools and flush larvae out of some sites, moderate rains distributed over the wet season probably prolong the life of temporary pools and facilitate more eggs and larvae to mature (Gillies and de Meillon, 1968). Studies in the East African highlands showed there was high spatial variation in the sensitivity of malaria outpatient numbers to climate fluctuations (Zhou *et al*., 2004). Rainfall was found to be significantly associated with the occurrence of *P. falciparum* malaria parasites (Imbahale *et al*., 2012). Seasonal variability in malaria transmission has been reported in Nigeria and other parts of Africa (Oyewole *et al*., 2007; Onyabe *et al*, 2003; Awolola *et al*., 2002).

Change in these climatic elements is expected to affect malaria indirectly by changing ecological relationships that are important to the organisms involved in malaria transmission (the vector, parasite, and host).The impact of these changes particularly on the distribution of *P. falciparum*

malaria has been projected by various groups (Rogers and Randolph, 2000; Hay *et al*., 2002) although little consensus is available (Patz *et al*., 2002; McMichael and Le Sueur, 2002). Evidence has shown that changes in temperature and precipitation have already changed the distribution and behaviour of the vector (Gebere-Mariam, 1984). In the last decade, malaria incidences seem to have significant association with meteorological variables (Alemu *et al.*, 2011). Thus, meteorological factors are important drivers of malaria transmission by affecting both malaria parasites and vectors directly or indirectly. Temperature, rainfall and humidity have been associated with the dynamics of malaria vector populations and, therefore, with spread of the disease.

CHAPTER THREE

MATERIALS AND METHODS

3.1 General methods

3.1.1 Study area

Anambra East Local Government Area (LGA) is one, out of the twenty one Local Government Areas (LGA) in Anambra State. It has its Headquarter in Otuocha. It is located between latitudes 6.16^0N to 6.58^0N and longitudes 006.49^oE to 006.93^0E. The altitudinal range of the LGA is 25m to 145m above sea level. Six towns / communities that make up the Local Government Area include Aguleri, Igbariam, Nando, Nsugbe, Umueri and Umuoba Anam. It is located in the tropical rainforest zone of the Southeastern Nigeria. The LGA has two marked climatic periods yearly. These are the wet and the dry seasons. Wet season takes place between April and October while the dry season occurs from November to March. The LGA has two large water bodies: Omambala and Ezu Rivers with their confluence in Aguleri. There are also other small water bodies, streams and large expanse of freshwater swamps.

The study area is largely rural and the inhabitants live in scattered clusters, that is typical of rural dwellings. According to NPC (2006), the estimated population of Anambra East LGA is 152, 159 (77,539 males and 74, 610 females). The inhabitants are mostly farmers and fishermen. Their major agricultural produces are yam, cassava, maize, rice, fish, banana, plantain and vegetables. Other inhabitants are traders, artisans, drivers, civil servants, students and other professionals. The economic activities in the LGA include trading, road and water transportation, house construction, farming, fishing, milling of rice and sand mining. Cattle are also herded by Fulani herdsmen around the communities in the study area.

Institutions of higher learning in the LGA include: Chukwuemeka Odumegwu Ojukwu University Igbariam Campus, Nwafor Orizu College of Education Nsugbe and Tansian University Teaching Hospital Aguleri. There are many secondary and primary schools in the LGA. Health facilities in the LGA include General Hospitals, Mission Hospital and Primary Health Centres. The LGA has four market days: Eke, Orie, Afor and Nkwo in line with other Igbo communities in the State.

Recently, oil and gas were found in large quantity in the area. This has resulted in massive construction activities especially the Cargo Airport at Umueri, and the consequent opening up of areas that were unoccupied. This factor has necessitated huge influx of people from different parts of Nigeria and beyond into the area. The map of Anambra State showing the location of Anambra East LGA (Figure 1) and the map showing the location of communities in Anambra East LGA (Figure 2) are shown below.

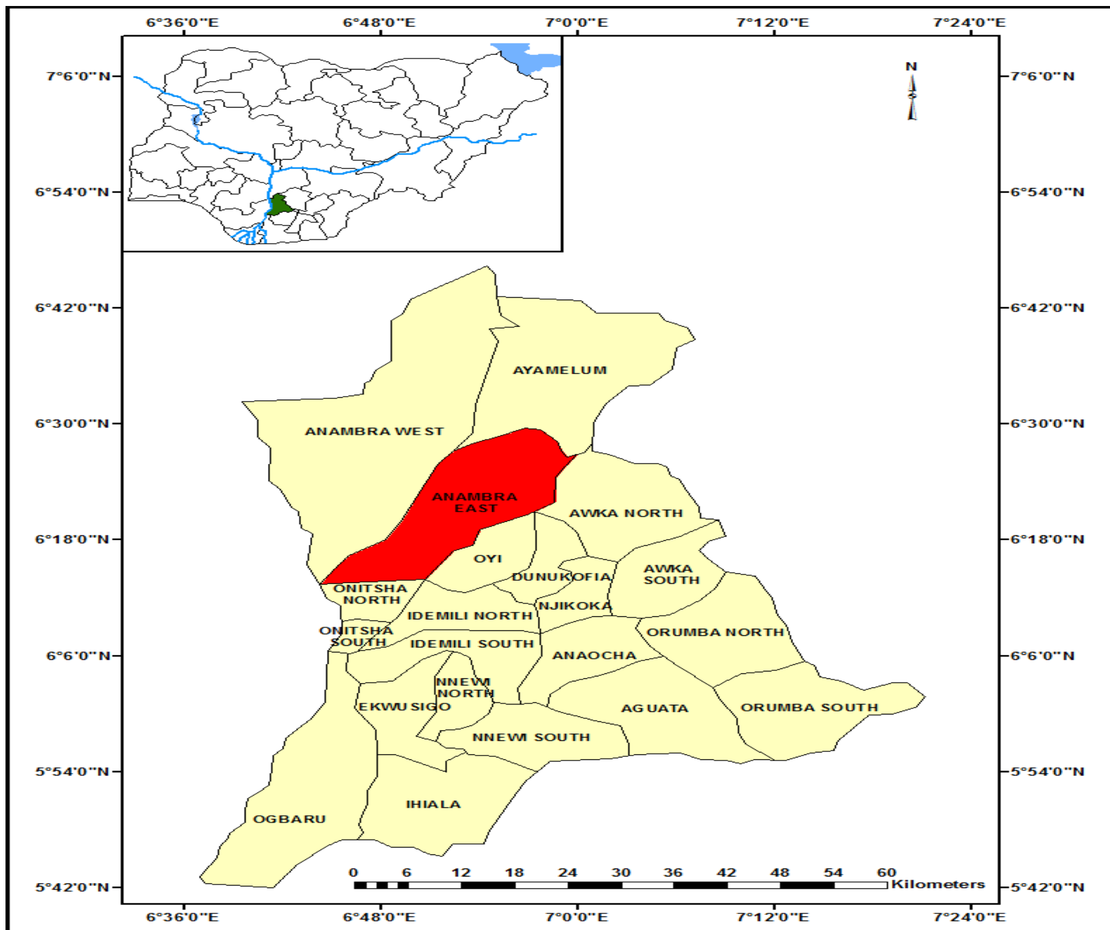
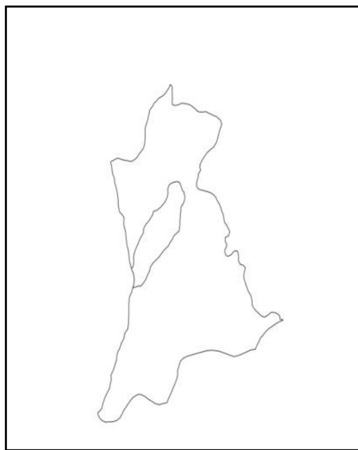


Figure 1: Map of Anambra State showing the location of Anambra East Local Government Area. Insert is the map of Nigeria showing the location of Anmabra State (Made by a cartographer at the Department of Geography, Nnamdi Azikiwe University, Awka).



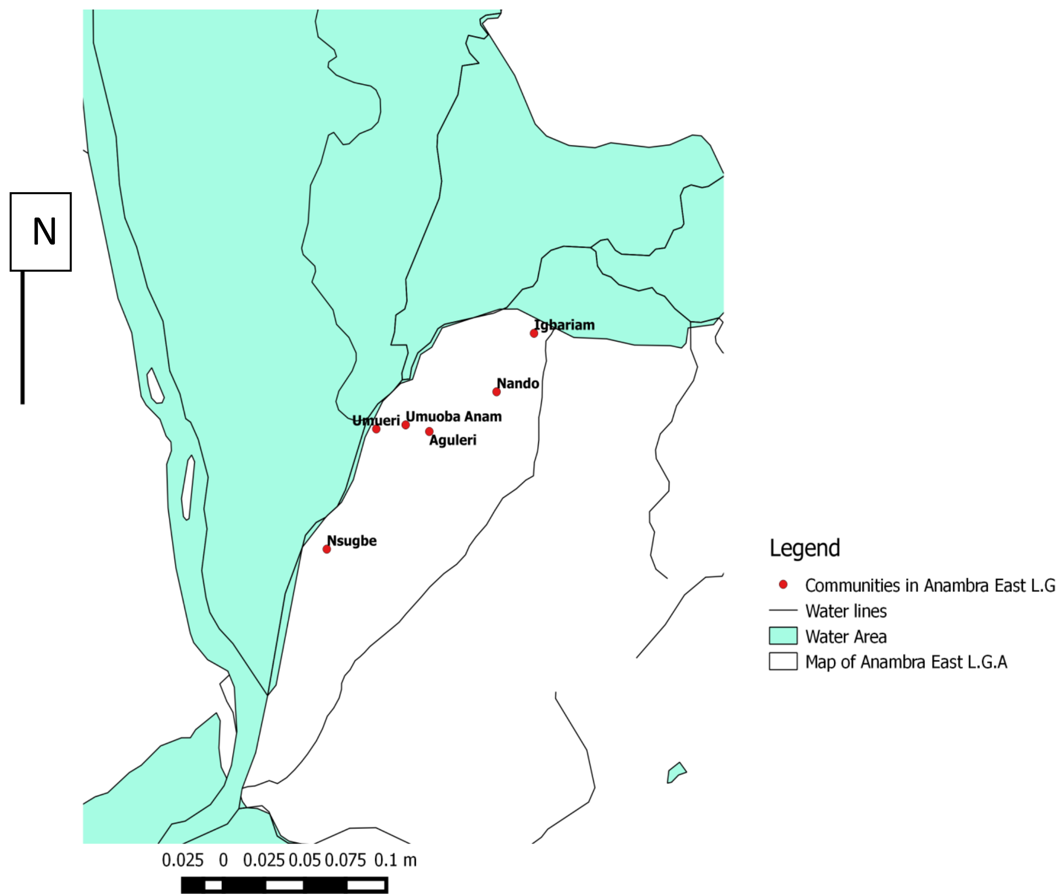


Figure 2: Map of Anambra East Local Government Area showing the location of the communities (using geographical coordinates of the communities with GIS version 2.10.1). Insert is the map of Anambra State showing the location of Anambra East Local Government Area.

3.1.2 Research design

The study entailed both parasitological and entomological survey. Field based longitudinal study that lasted for a period of 12 months (October 2016 to September 2017) was adopted for the collection of mosquitoes, climatic data and blood specimen. Morphological and molecular characterization of malaria vectors, physicochemical and biological characterization of the breeding sites as well as malaria parasite identification and quantification were laboratory based. Systematic sampling technique was used to select the communities included in the research. Along the longest stretch of roads that cut across the LGA, the 1st, 3rd and 5th (last) communities were selected. With that, Igbariam, Aguleri and Nsugbe were selected to represent the LGA. In each town, a total of ten (10) households according to WHO (2003) were systematically selected for monthly collection of both mature and immature stages of *Anopheles* mosquitoes.

Entomological survey was carried out to achieve objectives 1, 2, 3 and 4 as stated in Chapter one. In that regards, potential breeding sites of mosquitoes were surveyed for *Anopheles* mosquito arvae identification and collection. The water quality in the breeding sites was analyzed to characterize the breeding sites. Indoor and outdoor biting/resting adult Anopheles mosquitoes were collected within the chosen households using Pyrethrum Knockdown Collection (PKC) method and Human Bait Collection (HBC) Method respectively. All the females belonging to *Anopheles* species were identified, counted and classified on the basis of their abdominal conditions as unfed, freshly fed, half-gravid or gravid (WHO, 2003). Further, the females were preserved individually in an eppendorf tubes containing silica gels, and stored at room temperature for identification of cryptic species, determination of their infectivity rates and host preferences.

All buildings and sites sampled for mosquitoes in the three towns were identified and geo-referenced using a hand-held geographical positioning system (eTrex, Vista, Garmin, USA) (Figure 3).

Parasitological surveys was carried out to achieve objective 5. This was done simultaneously with entomological surveys. It involved the collection and examination of blood samples from apparently healthy individuals that inhabit the selected towns. The study participants were selected using convenient sampling technique. Prevalence and intensity of malaria parasite

infection among the study participants were determined and summarized by months and seasons of the year.

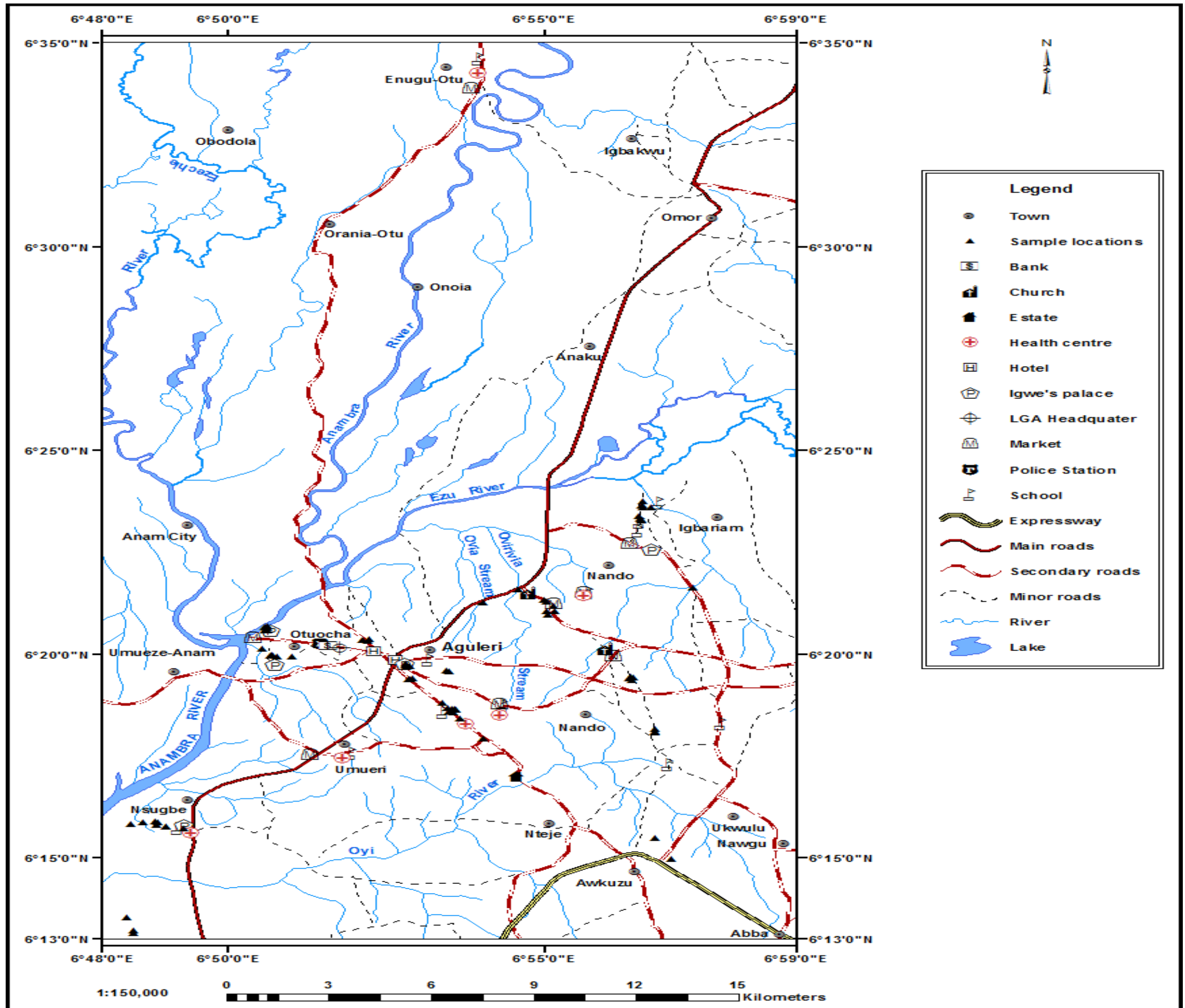


Figure 3: Map showing sample collection points in Anambra East Local Government Area. (Points were picked using a hand-held geographical positioning system)

3.1.3 Ethical Approval

The study proposal was presented in October, 2016 to the School of Postgraduate Studies, Nnamdi Azikiwe University, Awka for approval. Thereafter, ethical approval was obtained from the Ethical Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Amaku, Awka (Appendix 1).

3.1.4 Advocacy visits and community mobilization

Advocacy visits were made to Anambra East LGA Chairman with a Letter of Introduction from the Head of Parasitology and Entomology Department Nnamdi Azikiwe University, Awka, for permission to work in the area (Appendix 2). Visits were also made to the traditional rulers of the selected communities to obtain permission to work with their people. House to house sensitization was done in different villages prior to specimen collection. Volunteers involved in the collection of adult mosquitoes were educated on the nature of the field work and the implications. They were trained on how to collect landing mosquitoes prior to blood feeding to minimize the risk of malaria transmission. They were given prophylactic treatment for malaria 10 days before commencement of the studies according to Onyido *et al*., (2008). Informed consents were obtained from individuals whose blood specimens were collected. A medical Laboratory Scientists was employed to help in collection of the blood samples for parasitological investigations.

3. 2 Malaria vector species composition and the climatic factors influencing their survival and population abundance.

3.2.1 Malaria vector species composition

Mature and immature stages of *Anopheles* mosquitoes from the study area were collected and identified using the methods decribed below.

3.2.1.1 Collection and rearing of immature stages of *Anopheles* mosquitoes

Potential breeding sites of mosquitoes were surveyed from October 2016 to September 2017 in the selected communities WHO (2003). Collections of the larvae were made with a ladle and white plastic bowls. *Anopheles* larvae were identified in the field by their parallel positions on water surfaces as well as the characteristic sharp backward movement they exhibit WHO (2003). When collected, they were transferred into a well labeled plastic container and transported to the Laboratory unit of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka where they were reared to adult for proper identification and processing for further entomological studies.

Mosquito larvae from different breeding habitats were separately reared to adult for proper identification to species level according to WHO (2003). In the laboratory, larvae collected from different breeding sites were put into separate transparent plastic bowls of 2cm depth and labeled appropriately. The plastic bowls were covered with mosquito nettings held in position with rubber bands. This was a precaution to prevent emerging mosquitoes from escaping and also prevent wild mosquitoes from laying eggs into the container. The larvae were reared at room temperature and fed with a mixture of biscuits and yeast. They were closely monitored and the pupae were collected into different plastic cups using Pasteur pipette and placed in labeled cages for adult emergence.

3.2.1.2 Pyrethrum Knockdown Collection (PKC) of adult *Anopheles* mosquitoes

In each of the selected households, sleeping rooms were used for PKC according to World Health Organisation (2003) to determine *Anopheles* mosquito composition in indoor location. This was done very early in the morning between the hours of 6.00am and 8.00am immediately after the house occupants were awake. In doing that, all food materials were covered to avoid contamination with insecticide, small items of furniture were removed and openings and holes in the house were sealed with cloth or old newspapers. Then sheets of white cloth were spread so that they completely covered the floor and all flat surfaces of the remaining furniture. White sheets were also spread under tables, beds, and other places where mosquitoes normally hide. Commercially available pyrethroid insecticide, (Raid®) was sprayed in the room until the room

was filled with fine mist of the insecticide. Thereafter, the door was closed for 20 minutes. After that, the white sheets of cloth were picked up and taken outside, one at a time starting from the doorway. Knocked down mosquitoes were collected outside in the day light using forceps and transferred into a well labeled Petri dish lined with damp cotton wool and filter paper on top of the cotton wool. Collected mosquitoes were transported to the laboratory unit of Parasitology and Entomology Department, Nnamdi Azikiwe University Awka for morphological identification and subsequently to Vector Control Research Lab, Department of Public Health & Epidemiology, Nigeria Institute of Medical Research, Yaba Lagos for molecular identification and infectivity test (Appendix 3).

3.2.1.3 Human Bait Collection (HBC) of adult *Anopheles* mosquitoes

Human bait collection of adult mosquitoes was carried out to determine the *Anopheles* mosquito composition in outdoor locations, as well as their biting time. Human volunteers, serving as baits were used for the collection around the selected houses in the towns. Mosquito collections were made at hourly interval from 4.00pm to 6.00am local time. Ten volunteers were used for each collection to represent ten houses sampled with PKC. Mosquitoes were collected as soon as they settled on the exposed skin of the bait using a sucking tube and transferred to well labeled paper cups. Collected mosquitoes were also transported to the laboratory unit of Parasitology and Entomology Department, Nnamdi Azikiwe University Awka for morphological identification; and subsequently to Vector Control Research Laboratory, Department of Public Health and Epidemiology, Nigerian Institute of Medical Reseach, Lagos Nigeria for molecular identification as well as blood meal analysis and infectivity test

3.2.1.4 Morphological identification of *Anopheles* species

Identification of the adult mosquitoes that were raised through rearing of the immature stages and those collected through PKC and HBC, was done using the identification key by Gillett (1972) and Gillies and Coetzee (1987). Live mosquitoes were anaesthetized with chloroform and sexed before identification. Different *Anopheles* species were identified using the characteristic

banding patterns of their palps, the scales on their wings as well as their legs. Identified *Anopheles* species were preserved in a well labeled eppendorf tubes, one mosquito per tube.

3.2.1.5 Molecular identification of *An. gambiae* s. l.

Molecular identification of *An. gambiae* s. l. was done at the Vector Control Research Laboratory, Department of Public Health and Epidemiology, Nigerian Institute of Medical Reseach, Lagos Nigeria (Appendix 3). This was achieved by using rDNA Polymerase Chain Reaction (rDNA-PCR) of the *An. gambiae* s. l. intergenic spacer regions (IGS) and Agarose gel electrophoresis (Scott *et al.,* 1993).

3.2.1.5.1 Extraction of *An. gambiae* s. l. DNA and Polymerase Chain Reaction (PCR)

PCR amplification of deoxyribonucleic acid from *An. gambiae* s. l. was performed as described by Scott *et al.* (1993). Legs and wings of each *An. gambiae* s. l was placed in 1.5ml tube and ground with 200µl grinding buffer. This was incubated in heating block set at 70^{0}C for 30 minutes. To that, 28µl of 8M Potassium Acetate was added and mixed gently by tapping with fingers. This was incubated on ice for 30 minutes before centrifuging at 13,000 rpm for 20 minutes. The supernatant was transferred into a new tube by pipetting. There was addition of 400µl of 100% ice cold ethanol and another centrifugation for 30 minutes. The ethanol was removed by pipetting without disturbing the pellet. To the tube containing the pellet only, 200µl of 70% ice cold ethanol was added and centrifuged again for 15 minutes. The ethanol was decanted by pipetting and the tubes were kept on the bench with the tops open for the pellets to dry. The pellets were re-suspended in 100µl of 1X Tris-Borate Ethylene-di-amino tetra acetic acid (TBE) buffer, allowed to dissolve and stored at -20^{0}C.

The PCR chemicals which had been frozen at -200^{0}C was thawed and mixed to produce a master mix. Each component of the master mix plays specific role in the process. Buffer provides a suitable chemical environment for optimum activity and stability of the DNA polymerase.

dNTPs (nucleotides) are the building blocks of nucleic acids (DNA/ RNA). Primers (CAGACCAAGATGGTTAGTAT for *An. quadriannulatus*, AAGTGTCCTTCTCCATCCTA for *An. arabiensis*, TGACCAACCCACTCCCTTGA for *An. merus*, CTGGTTTGGTCGGCACGTTT for *An. gambiae* s. s. and GTGTGCCCCTTCCTCGATGT as Universal primer) which are short (20bp) single stranded nucleic acid (oligonucleotide), hybridized with a complementary portion of another molecule acts as starting points for strand synthesis with DNA polymerase. RTaq (*Thermus aquaticus*) or Taq DNA polymerase, an enzyme DNA polymerase synthesize new DNA strands using pre-existing DNA strands as templates. DNA polymerase (i.e. Taq) is a metallo-enzyme and therefore requires a source of divalent cation to function in which case $MgCl_2$ was used. Deionised water was used to get the final volume.

Twelve and a half microliter (12.5µl) of the master mix was added into 200µl tube and 1µl of extracted DNA of *An. gambiae* s. l was also added using micro pipette. The tubes were centrifuged for 2 minutes in a micro centrifuge at 16000 rpm for 15 minutes. Four positive and 1 negative controls were also prepared as in the specimen. One drop of mineral oil was added into each tube. The tubes were loaded in the PCR machine and run according to the selected program. At the end of PCR, the products now called APPLICORNS were brought out and agarose electrophoresis ran for them.

3.2.1.5.2 Agarose gel electrophoresis

Agarose gel electrophoresis was run for the applicorns generated from PCR (Scott *et al.*,1993). The 1.5% agarose gel was prepared and poured into electrophoretic chamber and allowed to solidify; care being taken to avoid air bubbles. Afterwards, the applicorns from the PCR machine were brought out. Then 10µl of each applicorn was mixed with small amount of Ficol dye on a plate. The dyed applicorns were introduced singly into the gel in the electrophoretic chamber, one applicorn in one well. The DNA ladder, was added too. DNA ladder contains different samples of known molecular weight, if the positive control bands are missed out, the degree of migration of DNA of mosquito species would be compared with known molecular weight of the ladder (because the molecular weight of mosquito species determines the degree of migration).

So the ladder serves as another positive control. The electrophoresis was run at 100V and not more than 120-150mA for 1 hour 10 minutes. In the end of the process, the gel picture was taken under UV light using gel documentation system. The bands were read and compared with the bands at DNA ladder results.

3.2.2 Determination of the climatic factors influencing the survival and population of *Anopheles* mosquitoes

This was achieved using secondary data obtained from Nigerian Meteorological Agency (NIMET) synoptic office located at Enugwu-Agidi, Njikoka LGA, Awka Capital territory, Anambra State (Appendix 4). Information on temperature (measured in ^{o}C), rainfall (measured in mm) and relative humidity (measured in %) were correlated with the abundance of adult *Anopheles* species.

3.3 Breeding ecology, physicochemical and biological factors operating in *Anopheles* mosquito habitat.

The breeding sites of different *Anopheles* mosquitoes were identified as described in section 3.2.1.1. The physical, chemical and biological characteristics of the identified breeding sites were determined using the methods described below:

3.3.1 Determination of the biological characteristics of the breeding sites

The biotic components of the breeding sites were determined both in the field and in the laboratory. In the field, the vegetation cover, vertebrate and invertebrate fauna present in the breeding habitats were observed and recorded. The microbial load of the water samples was determined according to Cheesbrough (2006) in the Laboratory Unit of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

3.3.1.1 Isolation and characterization of bacterial flora

The abundant bacteria and fungi present in the breeding habitats of *Anopheles* mosquitoes were isolated, characterized and identified in the laboratory (Cheesbrough, 2006). For bacterial organisms, the culture media used for this purpose was nutrient agar. The media was prepared

based on the manufacturer's instruction. Using a weighing balance, 12g of nutrient agar powder was weighed and poured into a conical flask. After, 250 ml of distilled water was poured into the flask and stirred. This was sterilized using autoclave at 121°C for 15 minutes. After cooling, the media was poured into Petri dishes sterilized using hot air oven at 160°C for 90 minutes. This was then allowed to solidify.

One milliliter of each water sample was spread into separate nutrient agar plates and incubated at 37°C for 24 – 48 hours. Each colony was observed on a plate and checked for its purity. The impure colony was subcultured by transferring it to nutrient broth and then streaking into nutrient agar plates for pure colony growth. Bacterial colonies were further purified and preserved on nutrient agar slants at 4°C for identification.

3.3.1.2 Identification of bacteria isolates

The bacteria isolates were identified using colony cultural characteristics, colony morphology and biochemical characterization. The cultural characteristics of bacterial isolates such as colony color, colony elevation, colony margin and colony surface were inspected visually in a plate. Morphological characteristics of bacterial isolates such as spore stain, gram staining, shape and motility tests were determined by light microscope when viewed using ×40 objective lens (Cheesbrough, 2006).

3.3.1.2.1 Gram staining procedure

A sterile wire loop was used to put a drop of normal saline at the center of grease – free slide. A portion of colony was picked and emulsified in the drop and allowed to air dry before heat fixing. Crystal violet was applied for 1 minute on the smear; it was later replaced with Lugol's iodine for another one minute before rinsing with distilled water. The slides were later flooded with 95% ethanol (decolouriser) for few seconds until the slides' appearance became free of violet stain. Slides were then rinsed with water and Safranin stain applied for 1 minute. This was followed by rinsing and air drying before being observed microscopically under × 100 objective lens in oil immersion. The bacterial cell that retained the purple color against the counter stain background indicated a Gram positive organism while the Gram negative organisms stained red.

3.3.1.2.2 Biochemical characteristics of the bacteria isolates

Biochemical characteristics of isolates, such as catalase test, coagulase test, indole test, motility test, oxidase test, and sugar fermentation test were carried out according to Cheesbrough (2006).

3.3.1.2.2.1 Catalase test

In catalase test, a drop of 3% hydrogen perioxide was put on a grease free slide. Portion of the colony of the test organism was picked and emulsified in it using a wire loop. This was examined for gas bubble which indicates catalase positive and the absence of gas bubbles indicates catalase negative. The test was used to differentiate between *Staphylococcus* species. The gas bubbles observed in the reaction was due to the breakdown of hydrogen peroxide to oxygen and water by enzymes called catalase.

3.3.1.2.2.2 Coagulase test

A drop of physiological saline was put on a clean glass slide, followed by making smear of 24 hours old isolate of test organism. Then a drop of human serum was added into it to make a suspension. Clumping indicates positive result which implies the ability of the test organism to produce coagulase, an enzyme that coagulate blood plasma while in a negative result no clumping was observed (Cheesbrough, 2006). This test is used to differentiate pathogenic *Staphylococcus aureus* from non-pathogenic staphylococci.

3.3.1.2.2.3 Indole Test

This test was done as described by Cheesbrough (2006); Miller and Wright (1982). It was carried out to determine the organisms that breakdown amino acid tryptophan into indole. Peptone water of 1.5g weight was dispensed into 250ml capacity conical flask. 100ml of distilled water was gradually added and shaken. This was finally dispensed into the test tubes and corked for sterilization in the autoclave at 121°C for 15minutes. A speck of each isolate was inoculated into 5ml of sterile peptone water and was incubated at 37°C for 48 hours. About 4 drops of Kovac's reagent was added into each tube and gently shaken. Positive test was indicated by a red colour that occured immediately at upper part of the test tube.

3.3.1.2.2.4 Motility test

The isolate was cultured in sterile peptone water medium in the test tube and incubated for 24 hours at 37°C. A drop of the suspension was placed on a cover slip and glass (concave glass slide) with plasticin made circular on it was inverted on the cover slip. This was carefully inverted and then observed for motility under ×40 objective lens.

3.3.1.2.2.5 Oxidase Test

This is used to determine the presence of oxidase enzyme. Filter papers were dipped into a freshly prepared oxidase reagent and allowed to dry for a little while. With a wire loop, a colony of the test organism from the culture plate was removed and smeared on the filter paper. A purple colour change of the organism immediately it is smeared on the filter paper indicated a positive result while purple colour change after 10-20 seconds indicated a negative result.

3.3.1.2.2.6 Sugar fermentation (lactose, maltose and fructose) test

This test is used to determine the ability of the organism to ferment a specific carbohydrate with or without the production of gas. Bromothymol blue was used as an indicator in the media. Fermentation of the carbohydrate produces acids, causing the media to change from blue to yellow. Inverted tubes called Durham tube, traps some of the gas the organism produces, allowing production to be seen (if it ferments, gas will be produced). Each of the test tubes containing the carbohydrate medium was inoculated with the test organisms and incubated at optimum temperature for 24 hours. When the media turned yellow (fermentation had occurred) and gas is produced, it indicated positive result and when it remained blue (no fermentation occurred) which indicated negative result.

3.3.1.2 Isolation of fungi from the breeding sites of mosquito

3.3.1.2.1 Preparation of Media

The media used was Sabouraud Dextrose Agar (SDA) for the isolation of fungal organisms. The media were prepared according to the manufacturer's instruction by weighing 65g of the powder and dissolving it in 1000ml of water. The solution was homogenized using the magnetic stirrer

and sterilization at 121°C for 15minutes was done using an autoclave. The prepared media was allowed to cool and poured into petri dishes.

3.3.1.2.2 Innoculation of samples on the plate.

The water samples were inoculated on the prepared SDA by streaking. This was incubated aerobically at 25°C for 3 days. Respective colonies formed were sub-cultured and incubated severally to get pure culture. After that, the plates were observed for colony morphology.

3.3.1.2.3 The slide culture technique

The slide culture technique according to Wijedasa, and Liyanapathirana (2012) was also employed. The SDA medium in the Petri dish was cut into square blocks, 1cm × 1cm with a sterile blade. An agar square was placed at center of the sterile slide on a sterile V shaped bent glass rod in a sterile Petri dish. A wire-tip of the culture was inoculated into the mid-point of each of the four edges of the agar square. The cover slip was lifted by means of sterile forceps and placed over the surface of the inoculated agar block. About 10ml of sterile distilled water was poured in the bottom of the Petri dish and the dish covered. The slide culture plates were incubated at room temperature for 3 -5 days. The growth was examined after 3 or 5 days. When sufficient growth occurred, stained preparation was made. The cover slip was removed and the agar block discarded without disturbing the rectangles of growth on the slide and cover slip. A drop of lacto-phenol blue was immediately added and the second sterile cover slip gently placed. Similarly, the growth on the cover slip was stained and placed on the second sterile slide. The two slides preparations were examined under the low (×10) and high (×40) power of the microscope. Observed features such as the hyphae and spore heads were recorded.

3.3.1.2.4 Identification of the fungal isolates

Both macroscopic and microscopic identification were carried out based on the criteria described by St-Germain and Summerbell (1996) and Kidd *et al.,* (2014). Macroscopically, the culture plates were observed from the third day for colonial morphological characteristics of the isolates.

Features observed were pigmentation, texture (dry, powdery, velvety, gelatinous) and colour of reverse of the plates. Microscopically, the features below were sought for after slide culture included: hyphae (septate or non-septate), mycelium (hyaline or coloured), type of asexual spores (sporangiospores, conidia, arthrospores or blastospores), appearance of conidia (shape, size (micro or macroconidia), colour, smooth or rough, one, two or many celled), characteristics of asexual spore-head, appearance of sporangiophores or conidiophores (simple or branched, and if branched, the type of branching), size and shape of the collumella at the tip of the sporangiophore, size and shape of the vesicle at the tip of the conidiophores, appearance of conidiophores (single or in bundle), presence of sexual spores (oospores, zygospores or ascospores) and Presence of special structures or spores (stolons, rhizoids, chlamydospores).

3.2.4.2.5 Biochemical test for yeasts identification

The suspected yeast-like organisms were identified by performing germ tube test as described by Sandven (1990). Three (3) drops of fresh pooled human serum were dispensed into 12 x 75 mm labeled test tubes using a Pasteur pipette. A colony of the isolate was picked with a sterile wooden applicator and emulsified in the serum. The stick was discarded in a discard container. The suspension was incubated at 37°C for 2 to 4 hours. A drop of the suspension was placed on a clean microscope slide. A clean cover glass was placed over the suspension and then examined with a microscope using the low power objective. High power objective was used to confirm the presence or absence of germ tubes. Controls were read and results recorded.

3.3.2 Determination of physicochemical characteristics of the various breeding habitats

The physicochemical characteristics of the various breeding habitats visited were determined according to the method used by Oyewole *et al.* (2009). Surface water temperature was determined at the site using mercury in glass thermometer. Water samples were then collected from the identified habitats using two 500 ml capacity specimen bottle for each breeding habitat. Water in one of the bottles was fixed with diluted nitric acid (HNO_3); whereas water in the other bottle ws not fixed. The water samples were transported in a dark covered plastic bucket to

Project Development Institute, Federal Ministry of Science and Technology (PRODA) Enugu and Natural Products Research and Development Laboratory, Nnamdi Azikiwe University, Awka for analysis to determine the pH, Salinity, Total Dissolved Solute (TDS), Total Suspended Solutes (TSS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Sulphate, Iron, Cadmium, Lead, Manganese and Phosphorous concentrations.

3.3.2.1 Determination of pH.

In the laboratory, the pH was determined using a pH meter. In doing that, the meter was switched on and the pH electrode put into the buffer solutions one at a time. A standby 250 ml capacity beaker was filled with water for rinsing the electrode before changing over from one buffer solution to another. The pH meter was calibrated with buffer 4.01, 7 and 9.21 by pressing the CAL button. When that was done, the pH of the water samples were determined by pressing the READ button.

3.3.2.2 Determination of Salinity

Salinity was measured using YSI moulting meter, salinometric method was the principle used to determine salinity.

3.3.2.3 Determination of Total Dissolved Solid (TDS)

Total Dissolved Solid (TDS) was measured in the laboratory by weighing an empty beaker first (Initial weight) and filtering 50 ml of the water sample into it. The beaker was heat to dryness, cooled and weighed (Final weight). The DS residue was calculated by subtracting the initial weight of the beaker from the final weight; and the TDS value given in mg/l using the formula below:

$$\text{TDS} = \frac{\text{DS residue} \times 1000}{\text{Volume of sample used}}$$

3.3.2.4 Determination of Total Suspended Solute (TSS)

In determining the Total Suspended Solute (TSS), an empty filter paper was weighed (Initial weight). This was used to filter the 50 ml of water for TDS. Then the filter paper containing residue was allowed to dry and reweighed (Final weight). The difference between the final weight of the filter paper and the initial weight was obtained and converted to mg/l using the same formula for TDS.

3.3.2.5 Determination of Chemical Oxygen Demand (COD)

To determine the Chemical Oxygen Demand (COD), two set-ups were used for each water sample; one for blank and the other for the test water. For the test, 10 ml of the water sample was pipetted into a beaker while for the blank; 10 ml of distilled water was pipetted into a beaker. In each of the beakers, 5 ml of 0.025N potassium dichromate ($K_2Cr_2O_7$), 15 ml of concentrated sulphuric acid (H_2SO_4) and 40 ml of distilled water were added sequentially to get a 70 ml solution. When acid was added, the solution became hot. Then 7 drops of phenanthroline ferrons sulphate indicator (Ferroin indicator) was added and the solutions were allowed to cool. In a burette, 0.025N Ferron Ammonium sulphate was used to titrate the solutions until the colour changed from greenish blue to orange.

The COD was then calculated using the formula;

$$\frac{(T_1 - T_2) \times 0.025N \times 5000}{\text{Volume of sample used}}$$

Where T_1 = titre value for blank

T_2 = titre value for water sample

N = normality for ferrons ammonium sulphate used which is 0.025

3.3.2.6 Determination of Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD).

A dissolved oxygen (DO) meter was used to obtain the biochemical oxygen demand (BOD) in each water sample. First, the initial dissolved oxygen concentration (mg/l) in each sample was measured. Each sample was then placed in a dark incubator at 20^0C for five days. After five days ± 3 hours, the DO meter was used again to measure a final dissolved oxygen concentration (mg/l). The final DO reading was then subtracted from the initial DO reading and the result is the BOD concentration (mg/l).

3.3.2.7 Determination of Sulphate concentration

To determine the Sulphate concentration, 10 ml of water sample was pipetted into a conical flask with 5 ml of 2M HCl (Hydrochloric acid) and 2 ml of 0.05M BaCl (Barium chloride) added before boiling for 5minutes. After cooling, 2 ml of ammonia and 5 ml of 0.01N EDTA were added and the solution boiled for 5 minutes. Using 5 ml of buffer 10 and 3 drops of Eriot (solochrome Black T) indicator, the solution was titrated with 0.01M $MgCl_2$. Colour change was observed from deep blue to light purple. Calculation of sulphate concentration was done using the formula: [10- (TV x 0.93)] x 96.01464 mg/l. Where TV= titre value of sample and 96.01464 is the molecular weight of sulphate.

3.3.2.8 Determination of Heavy metals

The water samples were first boiled at 100ºC. It was filtered to remove clogs, and then allowed to cool. They were then poured into a vacuum vial and placed in an Atomic Absorption Spectrophotometer. Using various wavelengths of metals, the concentration of iron, cadmium, lead, manganese and phosphorus were read and the values recorded.

3.4 Biting and resting behavior of the adult *Anopheles* mosquitoes to identify their preferred biting and resting places.

Pyrethrum Knockdown Collection (PKC) and Human bait collection (HBC) methods as described in sections 3.2.1.2 and 3.2.1.3 respectively were used to collect adult *Anopheles* mosquitoes. All females *Anopheles* species collected were classified based on their gonotrophic states as: blood-fed (engorged), gravid, half-gravid, or unfed (not engorged) according to WHO (2012).

3.4.1 Biting time.

Biting time was determined from hourly outdoor collections of *Anopheles* mosquitoes using Human Bait Collection method; between 4.00pm and 6.00am and for 12 months. Hourly biting time and monthly biting time were then calculated (Kabbale *et al.,* 2013).

3.4.2 Biting location

Biting location was determined from both the Pyrethrum Knockdown Collections and Human Bait Collections. PKC was used to estimate Man-biting Rate (Ma) indoors while HBC was used to estimate Man-biting Rate outdoors according to WHO (2012).

Indoor Ma (Ma_i) = Total number of blood fed female *Anopheles* species ÷ Total number of occupants that slept in the rooms used for PKC.

Outdoor Ma (Ma_o) = Total number of female *Anopheles* species caught by HBC ÷ Total number of collectors.

Endophagic index (ENGI) and Exophagic index (EXGI) were then calculated as follows:

$ENGI = [Ma_i / (Ma_i + Ma_o)] \times 100$

$EXGI = [Ma_o / (Ma_i + Ma_o)] \times 100$

3.4.3 Post feeding resting location

Post feeding resting location was determined from the Pyrethrum Knockdown Collections using the concept of Müller *et al*. (2017) on house entry and house exit behavior of *Anopheles* mosquitoes and WHO index (WHO, 2012). Estimated proportions of *Anopheles* mosquitoes resting indoors after blood meal (P_i) were obtained from the formular:

P_i = [(Mean number of gravid + half gravid females) ÷ mean number of blood fed females] × 100.

$P_o = 100 - P_i$, Where P_o is the estimated proportions of *Anopheles* mosquitoes resting outdoors

3.5 Entomological indices (*Anopheles* species human blood index, sporozoite rate and Entomological Innoculation Rate) of malaria transmission in the study area.

3.5.1 Human Blood Index (HBI)

The head and thorax of each mosquito were separated from the abdomen using a sharp blade. After each specimen, the blade and forceps were washed 3 times in wash solution, dipped once in 1M NaOH solution and wiped dry ready for next specimen. Abdomens were placed back to

the eppendorf tube for use in the determination of HBI. Head and thorax of each mosquito were placed together in another eppendorf tube and preserved for use in sporozoite rate determination.

Blood meal identification was carried out to determine the sources of *Anophele*s mosquito blood meal (human or non-human host preference). Blood meals of engorged female *Anopheles* were subjected to direct ELISA using phosphatase conjugate of anti human IgG (Fab specific). It was used to identify human blood based on the procedures of Beier *et al.* (1987). Each of the separated abdomen of blood fed *Anopheles* species was ground with 100µl of Phosphate Buffer Saline (PBS) in 1.5ml eppendorf tube to prepare mosquito titurates. A positive control was prepared using 10µl of human serum and 500µl of PBS. Into separate wells of microtitre plates, 50µl of the mosquito titurates as well as the positive control were added and incubated for 1 hour. After that, 200µl of PBS-Tween 20 (1L of PBS + 500µl of Tween 20) was used to wash the microtitre plate. There was addition of 50µl of prepared enzyme conjugate solution of anti human IgG. This was allowed to incubate for one hour before washing three times with 200µl PBS-Tween 20. After, 100µl of ABTS peroxidase substrate was added and incubated for 30 minutes. This was again washed three times with 200µl PBS-Tween 20 before adding 50µl of phosphatase substrate to each well. There was final incubation for 5 hours before the absorbance was read at 414nm. The proportion of mosquitoes with human blood (referred to as the human blood index, HBI) was calculated by:

$$HBI = \frac{\text{Number of Mosquitoes with Human Blood}}{\text{Total Number of Mosquitoes with blood}}$$

3.5.2 Sporozoite Rate (SR) determination

Preserved head and thorax of the *Anopheles* mosquitoes were screened to detect *P. falciparum* circumsporozoite (CS) proteins by using “sandwich” ELISA procedure according to Wirtz *et al.* (1987). The reagents used included: Phosphate Buffered Saline (PBS), Blocking Buffer (BB), Grinding Buffer (BB-NP40) and wash solution PBS-TWEEN 20.

Head and thorax of each mosquito specimen was ground in a 1.5ml tube with 50µl BB-NP40 and the pestle was rinsed with 150µl BB, making the solution inside the specimen tube to make a

total volume of 200µl; this is mosquito titurate. The pestle was washed 3 times in wash solution, dried to avoid contamination and ready for use.

One micro liter of monoclonal antibody (mAb) stock was diluted in 125µl PBS to give a 0.40µl/50µl solution of *P. falciparum* antibody. Fifty microlitre (50µl) of this solution was introduced into each well of the ELISA microtitration plate. The plate was covered and incubated for 30 minutes so that the mAb is adsorbed on the plate.

After the capture mAb has bound to the plate, the well contents are aspirated. The well was filled with BB (on 200µl/ well, approx. 20ml BB/plate) and incubated for 1 hour at room temperature so that the remaining binding sites of the mAb are blocked.

Mosquito samples were loaded at this stage. Fifty microliter (50µl) mosquito titurate was added per well. Positive and negative controls (positive control is a known sporozoite of *P. falciparum* while a negative control is titurate from an unfed mosquito) were also added. The whole mixture was covered and incubated for 2 hours at room temperature.

The wells were washed 3 times with PBS-TWEEN 20. Fifty microlitre (50µl) of peroxidase – linked mAb is then added to the wells. After about 1 hour, the well contents were aspirated and the plate was washed again. Fifty microlitre (50µl) of clear peroxidase substrate solution (mixture of ABTS (2, 2 azinodi-3-ethyl-benzthiazoline sulphate) (solution A) and hydrogen peroxide (Solution B) at a 1: 1 ratio) was then added per well and colour should change within few minutes; if positive it turns dark green. The colour change was read visually and at 405nm, 30 to 60 minutes after adding substrate. The sporozoite rate will be calculated thus: $x/N \times 100 =$ sporozoite rate (%). Where x = number of mosquitoes that contain sporozoites and N = total number of mosquitoes examined (WHO, 2012).

3.5.3 Entomological Innoculation Rate (EIR)

This was done by first, calculating the man-biting rate (M) from *Anopheles* species collected indoors using PKC. The man-biting rate (per night) was obtained by dividing the total number of fed mosquitoes (F) by the total number of human occupants (W) who spent the night in the

houses used for collection (WHO, 2003). M = F/W. EIR was finally calculated using the formular: EIR = [Man-biting Rate (M) × Sporozoite rate (S)] / 100.

3.6 Malaria endemicity through prevalence / intensity studies of malaria in the study area.

3.6.1 Study population and sample size computation

The study population included apparently healthy male and female individuals of different age groups and different social classes inhabiting the study area. Individuals who had taken any antimalarial drugs within two weeks of the sample collection were excluded from the study. This was to rule out the effect of the drug on the outcome of malaria parasite test. The number of participants in the study was determined using the sample size calculation formula for categorical data by Cochran (1977).

$$n_0 = \frac{(t)^2 * (p)(q)}{(d)^2}$$

Where t = value for selected alpha level of 0.25 in each tail = 1.96
p = prevalence = 0.67 (based on the prevalence of 67.0% of an ealier report in the study area).
q = 1- P = 0.23
d = acceptable margin of error for proportion being estimated = 0.05
n_0 = required minimum sample size. Based on that, a minimum sample size of 236 was calculated.

3.6.2 Collection of blood specimens

Blood specimens were collected with the help of a medical laboratory scientist mainly by venepuncture (Cheesbrough, 2009) so that some quantity of each specimen is left for repeating bad slide preparations. A tourniquet was tied around the upper arm in order to make the veins prominent as well as increase blood pressure in the vein. The area from where the needle was inserted into the body was cleaned thoroughly using a cotton swab moistened with 70% alcohol. The needle was then inserted into the vein and 1ml of blood drawn into the syringe. The touniquette was loosened before the needle was pulled out from the vein. The blood was put in a well labeled EDTA (Ethylene Di amine Tetra Acetic Acid) bottle and mixed thoroughly to avoid clotting.

In some cases where the veins were hard to locate, finger prick method was used and the capillary blood expressed into EDTA containers.

3.6.3 Preparation of thick and thin blood smears

New, clean and grease-free slides were used for preparing blood films for microscopy. Both thick and thin blood films were made on the same slide for the detection of malaria parasite life cycle stages and identification of the *Plasmodium* species present respectively. Using a micropipette, 5 µl of blood was placed on the center of a slide and another larger drop of blood, 6 µl was placed to the right (WHO, 2015). The smaller drop of blood was spread using a smooth edged slide spreader to make a thin film. Without delay, the end of a plastic bulb pipette was used to spread the larger drop of blood until a circle of about 12 mm diameter is evenly covered for the thick smear. The blood films were then allowed to air-dry on flat surfaces.

3.6.4 Staining of the thick and thin blood smear

Staining of the blood films was done using 10% v/v Giemsa stain. Prior to that, the thin blood films were fixed by dipping that end of the slide in absolute methanol for few seconds. After that, the slides were placed in a rack, at acute angle so that the film-side faced upward and the thin film occupied the downward position. This was to prevent the thick film from being stained by methanol fumes and run-off. The slides were stained by flooding each slide separately with the diluted Giemsa stain to cover the thick and the thin blood films. This was allowed to stand for 10 minutes (Cheesbrough, 2009). After that, the slides were rinsed by flooding them with gentle flow of water until the stains was removed. The back of each slide was cleaned and placed in a draining rack for the preparation to dry.

3.6.5 Examination of the blood films

Both blood films were examined microscopically using 100x oil immersion objective lens. For each slide, the thick blood film was examined first in order to detect the presence of sexual and asexual stages of malaria parasites. This was followed by the examination of the thin blood film

for identification of the *Plasmodium* species present according to Cheesbrough (2009) and WHO (2015).

3.6.6 Determination of malaria parasite intensity

Detection of malaria parasites was performed against a standard number of White Blood Cells (WBC) on the thick film (WHO, 2015). The malaria parasites were counted alongside WBCs in the stained thick films. Malaria parasites were counted against 200 leucocytes and converted to number of parasites per microlitre (µl) by assuming a standard leukocyte count of 8,000/µl (WHO, 2015). A slide is declared negative if no parasites are seen after reading the set WBCs. The final number of parasite per microliter of blood was calculated using the formular:

Parasite/µl of blood = (Number of parasites counted × 8000 WBC) / 200 WBC

3.6.7 Data summary and statistical analysis

Data collected were summarized using tables, graphs and charts. Test of statistical significance was conducted using Chi square, ANOVA, Correlation, T – test and Friedman test at 5% level. Friedman test was used to compare the productivity of *Anopheles* species breeding sites. Correlation analysis was used to compare the abundance of mosquito larvae with the different physical and chemical parameters of the breeding sites. It was also be used to show the relationship between the following: *Anopheles* species abundance and malaria prevalence, prevalence of malaria parasite and sporozoite rate of *Anopheles* species, intensity of malaria parasite infection and sporozoite rate of *Anopheles* species, Entomological Innoculation rate and prevalence of malaria parasite, Entomological Innoculation rate and intensity of malaria parasite infection, and the effect of climatic data on the prevalence, intensity, sporozoite rate. ANOVA was used to compare the overall monthly and seasonal abundance, human blood index, sporozoite rates and Entomological inoculation rates of *Anopheles* mosquitoes. Paired T test was used to compare the endophagic and exophagic indices of *Anopheles* mosquitoes. Chi square test was used to show if the prevalence of malaria in the study area was dependent on variables like location, seasons and months of the year. Independent sample T test was used to compare the wet and dry season mean malaria parasite intensities. The statistical package used was SPSS version 25.0.

CHAPTER FOUR

RESULTS

4.1 Malaria vector species composition and the climatic factors influencing their survival and population.

4.1.1 Malaria vector species composition and abundance.

A total of 8181 female *Anopheles* mosquitoes consisting of 4127 (50.4%) larvae and 4054 (49.6%) adults were collected in the study area (Table 1). There was no significant difference in the abundance of larvae and adult *Anopheles* species collected (P = 0.893; Appendix 5). Four *An.* species namely *An. gambiae* s. l; 5,734 (70.1%), *An.* funestus; 1493 (18.2%), *An. moucheti*; 513 (6.3%) and *An. nili*; 441 (5.4%) were identified in the study area. There was significant difference in the abundance of different *Anopheles* species collected from the study area (P = 0.000; Appendix 6). Of the 1430 *An. gambiae* s. l. subjected to PCR, 56.2% had amplified rDNA IGS regions and only *An. gambiae* s. s. (100%) was identified (Plates 1a and 1b).

Table 1: *Anopheles* species composition and abundance in the study area.

Anopheles species	Adult		Larvae		Grand Total
	Abundance	Mean±SE	Abundance	Mean±SE	
An. gambiae s. s	3240	1080.0±135.0	2494	831.3±34.3	5734
An. funestus	492	164.0±16.7	1001	333.7±11.8	1493
An. moucheti	142	47.3±4.9	371	123.7±14.0	513
An. nili.	180	60.0±12.6	261	87.0±14.0	441
Total	**4054**	**1351.3±167.9**	**4127**	**1375.7±30.3**	**8181**

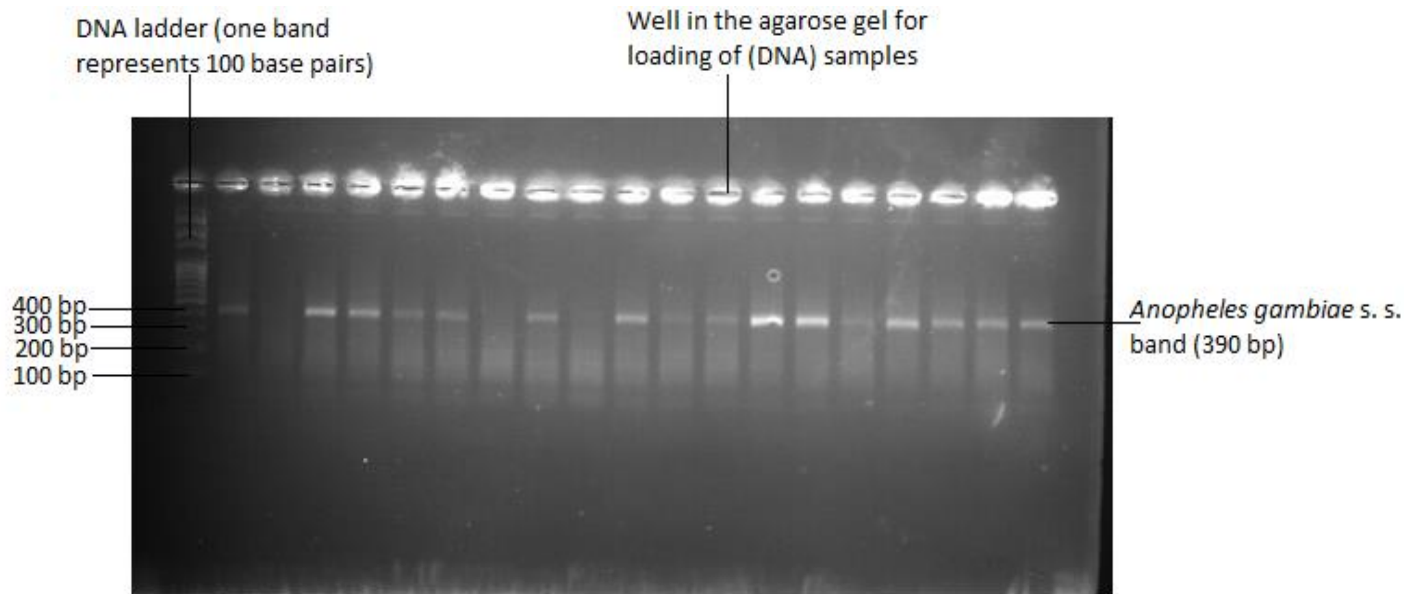


Plate 1a: Gel Image of *Anopheles gambiae* s. s.

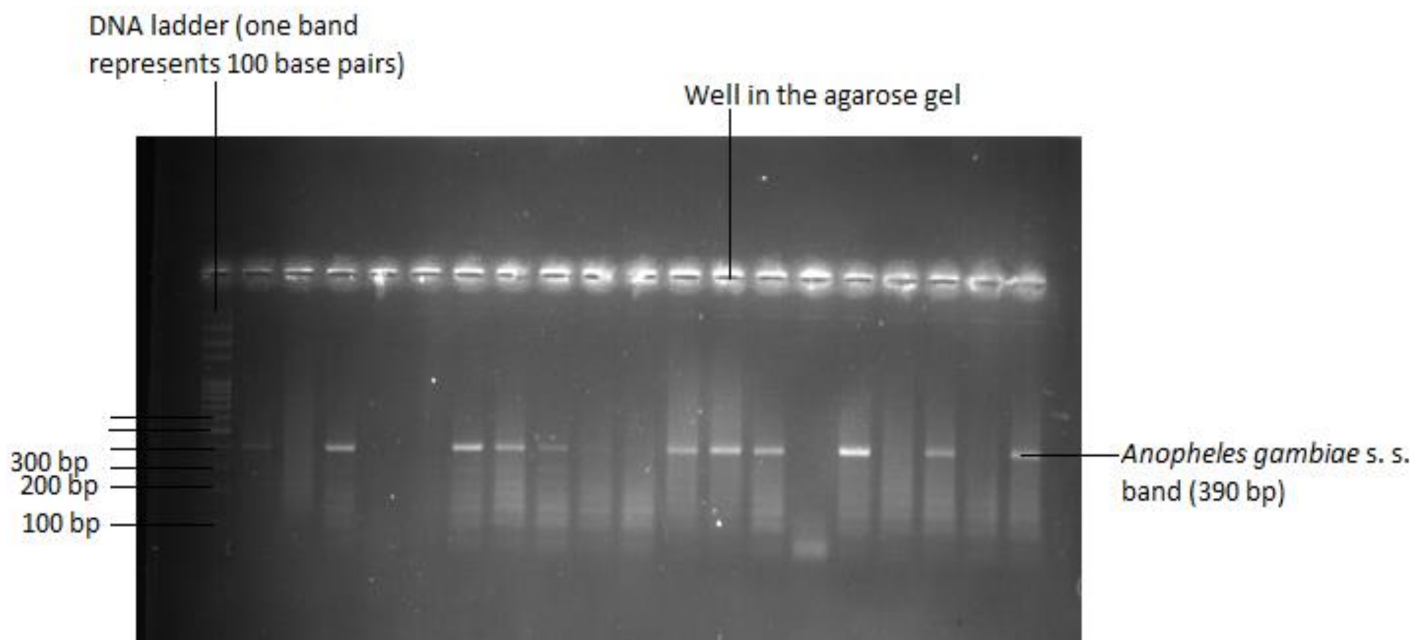


Plate 1b : Gel Image of *Anopheles gambiae* s. s.

4.1.2 *Anopheles* species composition and abundance in the communities selected from the study area.

Of the 8181 *Anopheles* mosquitoes collected from the study area, 2608 (31.9%) were collected from Aguleri, 3025 (37.0%) from Igbariam and 2548 (31.1%) from Nsugbe (Table 2).

Across the rows in Table 2, of the 5734 *An. gambiae* s. s. collected from the study area, 32.8% were collected from Aguleri, 36.6% from Igbariam and 30.5% from Nsugbe. Of the 1493 *An. funestus* collected from the study area, 31.3% were collected from Aguleri, 35.2% from Igbariam and 33.4% from Nsugbe. Of the 513 *An. moucheti* collected from the study area, 29.6% were collected from Aguleri, 40.4% from Igbariam and 30.0% from Nsugbe. Of the 441 *An. nili* collected from the study area, 23.8% were collected from Aguleri, 43.5% from Igbariam and 32.7% from Nsugbe.

Down the columns in Table 2, of the 2608 *Anopheles* mosquitoes collected from Aguleri, 72.2% were *An. gambiae* s. s., 17.9% were *An. funestus*, 5.8% were *An. moucheti* and 4.0% were *An. nili*. Of the 3025 *Anopheles* mosquitoes collected from Igbariam, 69.4% were *An. gambiae* s. s., 17.4% were *An. funestus*, 6.8% were *An. moucheti* and 6.3% were *An. nili*. Of the 2548 *Anopheles* mosquitoes collected from Nsugbe, 68.7% were *An. gambiae* s. s., 19.6% were *An. funestus*, 6.0% were *An. moucheti* and 5.7% were *An. nili*.

The diversity and dominance indices of *Anopheles* species collected from different communities in the study area are shown in Table 3. In Aguleri, the Simpson's index (D) of the *Anopheles* species was 0.56, Simpson's index of diversity (1 – D) of 0.44 and Simpson's reciprocal index (1/D) of 1.78. In Igbariam, the Simpson's index (D) of the *Anopheles* species was 0.52, Simpson's index of diversity (1 – D) of 0.48 and Simpson's reciprocal index (1/D) of 1.92. In Nsugbe, the Simpson's index (D) of the *Anopheles* species was 0.52, Simpson's index of diversity (1 – D) of 0.48 and Simpson's reciprocal index (1/D) of 1.92. The Simpson's reciprocal index (1/D) in all the three communities was approximately 2.

Anopheles mosquitoes collected from Nsugbe recorded highest value, 1.3291 for Shannon-wiener diversity (H) than Igbariam with H – value of 0.9162 and Aguleri with H – value of 0.8385. However, all the H – values in the study communities are approximately 1.

Table 2: ***Anopheles*** **species distribution and abundance in the communities.**

Anopheles **species**	**Selected communities**			**Total**	**Mean±SE**
	Aguleri	**Igbariam**	**Nsugbe**		
An. gambiae s. s	1883	2100	1751	5734	1911.3±101.7
An. funestus	468	526	499	1493	497.7±16.8
An. moucheti	152	207	154	513	171.0±18.0
An. nili.	105	192	144	441	147.0±25.2
Total	**2608**	**3025**	**2548**	**8181**	**2727.0±150.0**
%	**31.9%**	**37.0%**	**31.1%**	**100.0%**	

Sorenson's Coefficient (CC) of species similarity in the communities = 1.

Table 3: Dominance indices for *Anopheles* species collected from Anambra East LGA

Community	*An.* species	Abundance	Shannon-Wiener Diversity Index ; H = ∑ -pi(ln pi)					Simpson's Diversity Index; 1- D = 1-∑ n(n-1)/N(N-1)		
		n	**n/N (pi)**	**Pi^2**	**ln pi**	**pi(ln pi)**	**H**	**n-1**	**n(n-1)**	**D**
Aguleri	*An. gambiae* s. s	1883	0.7220	0.5213	-0.3257	-0.2352		1882	3,543,806	**1-D = 1-[(3,796,234)/(6,799,056)]**
	An. funestus	468	0.1794	0.0322	-1.7181	-0.3082		467	218,556	**1-D = 0.44**
	An. moucheti	152	0.0583	0.0034	-2.8422	-0.1657	**H = 0.8385**	151	22,952	**1/D = 1.78**
	An. nili	105	0.0403	0.0016	-3.2114	-0.1294		104	10,920	
	∑	N =2608	1.0000	0.5585	-8.0974	**-0.8385**		2607	6,799,056	
Igbariam	*An. gambiae* s. s	2100	0.6942	0.4819	-0.3650	-0.2534	**H = 0.9162**	2099	4,407,900	**1-D = 1-[(4,763,364)/(9,147,600)]**
	An. funestus	526	0.1739	0.0302	-1.7493	-0.3042		525	276,150	**1-D = 0.48**
	An. moucheti	207	0.0684	0.0047	-2.6824	-0.1835		206	42,642	**1/D = 1.92**
	An. nili	192	0.0635	0.0040	-2.7567	-0.1751		191	36,672	
	∑	3025	1.0000	0.5208	-7.5534	-0.9162		3024	9,147,600	
Nsugbe	*An. gambiae* s. s	1751	0.6872	0.4722	-0.7504	-0.5157	**H = 1.3291**	1750	3,064,250	**1-D = 1-[(3,356,906)/(6,489,756)]**
	An. funestus	499	0.1958	0.0383	-1.6307	-0.3193		498	248,502	**1-D = 0.48**
	An. moucheti	154	0.0604	0.0036	-2.8068	-0.1695		153	23,562	**1/D = 1.92**
	An. nili	144	0.0565	0.0032	-5.7446	-0.3246		143	20,592	
	∑	2548	1.0000	0.5173	-10.9325	-1.3291		2547	6,489,756	
Total	*An. gambiae* s. s	5734	0.7009	0.4913	-0.3554	-0.2491	**H = 0.8906**	5733	32,873,022	**1-D = 1-[(35,557,274)/(66,920,580)]**
	An. funestus	1493	0.1825	0.0333	-1.7010	-0.3104		1492	2,227,556	**1-D = 0.47**
										1/D = 1.9
	An. moucheti	513	0.0627	0.0039	-2.7692	-0.1737		512	262,656	
	An. nili	441	0.0539	0.0029	-2.9206	-0.1574		440	194,040	
	∑	8181	1.0000	0.5314	-7.7462	-0.8906		8180	66,920,580	

n = abundance of each *Anopheles* species, N = Total number of *Anopheles* species in a community, pi = proportion of a given *Anopheles* species in a community, ln pi = Natural logarithm of the proportion of a given *Anopheles* species in a community.

4.1.3 Monthly and seasonal abundance of *Anopheles* mosquitoes in the study area.

The monthly and seasonal abundance of the *Anopheles* mosquitoes are shown in Figures 4a and 4b. Out of the 8181 *Anopheles* mosquitoes collected from the study, the monthly mean abundance was 681.8±54.9**.** The highest number, 934 (11.4%) was collected in the month of May while the least number, 387 (4.7%) was collected in the month of December (Figure 4a). There was no significant difference in the monthly abundance of *Anopheles* mosquitoes in the study area (P = 0.968, Appendix 7). Seasonal abundance (Figure 4b) shows that 5473 (66.9%) *Anopheles* mosquitoes were collected in the wet season whereas 2708 (33.1%) were collected in the dry season. There was significant difference in the seasonal abundance of *Anopheles* species (P = 0.022; Appendix 8).

The monthly mean and seasonal abundance of different *Anopheles* species are shown in Figures 5a and 5b. *An. gambiae* s. s. recorded the highest monthly mean abundance of 477.8±38.9 whereas *An. nili* recorded the least monthly mean abundance of 36.8±4.4 (Figure 5a). There was significant difference in the monthly abundance of different *Anopheles* species collected from the study area (P = 0.000; Appendix 9).

Out of the 5734 *Anopheles gambiae* s. s. collected from the study, the monthly mean abundance was 477.8±38.9**.** The highest number, 667 (11.6%) was collected in the month of May while the least number, 267 (4.7%) was collected in the month of December (Figure 5a). There was no significant difference in the monthly abundance of *An. gambiae* s. s. (P = 0.169; Appendix 10). Seasonal abundance shows that 3901 (67.9%) *An. gambiae* s. s were collected in the wet season whereas 1833 (32.1%) were collected in the dry season (Figure 5b). There was significant difference in the seasonal abundance of *An. gambiae* s. s (P = 0.007; Appendix 11).

Out of the 1493 *An. funestus* collected from the study, the monthly mean abundance was 124.4±12.8**.** The highest number, 180 (12.1%) *An. funestus* was collected in the month of March while the least number, 64 (4.3%) was collected in the month of October (Figure 5a). There was no significant difference in the monthly abundance of *An. funestus* (P = 0.610, Appendix 12). Seasonal abundance shows that 989 (66.2%) *An. funestus* were collected in the wet season whereas 504 (33.8%) were collected in the dry season (Figure 5b). There was no significant difference in the seasonal abundance of *An. funestus* (P = 0.123; Appendix 13).

Out of the 513 *An. moucheti* collected from the study, the monthly mean abundance was 42.8±3.8**.** The highest number, 60 (11.7%) *An. moucheti* was collected in the month of June while the least number, 24 (4.7%) was collected in the month of November (Figure 5a). There was no significant difference in the monthly abundance of *An. moucheti* (P = 0.303, Appendix 14). Seasonal abundance shows that 306 (59.6%) *An. moucheti* were collected in the wet season whereas 207 (40.4%) were collected in the dry season (Figure 5b). There was no significant difference in the seasonal abundance of *An. moucheti* (P = 0.780; Appendix 15).

Out of the 441 *An. nili* collected from the study, the monthly mean abundance was 36.8±4.4**.** The highest number, 60 (13.6%) *An. nili* was collected in the month of April while the least number, 19 (4.38%) was collected in the month of December (Figure 5a). There was no significant difference in the monthly abundance of *An. nili* (P = 0.333, Appendix 16). Seasonal abundance shows that 277 (62.8%) of *An. nili* were collected in the wet season whereas 164 (37.2%) were collected in the dry season (Figure 5b). There was no significant difference in the seasonal abundance of *An. nili* (P = 0.475; Appendix 17).

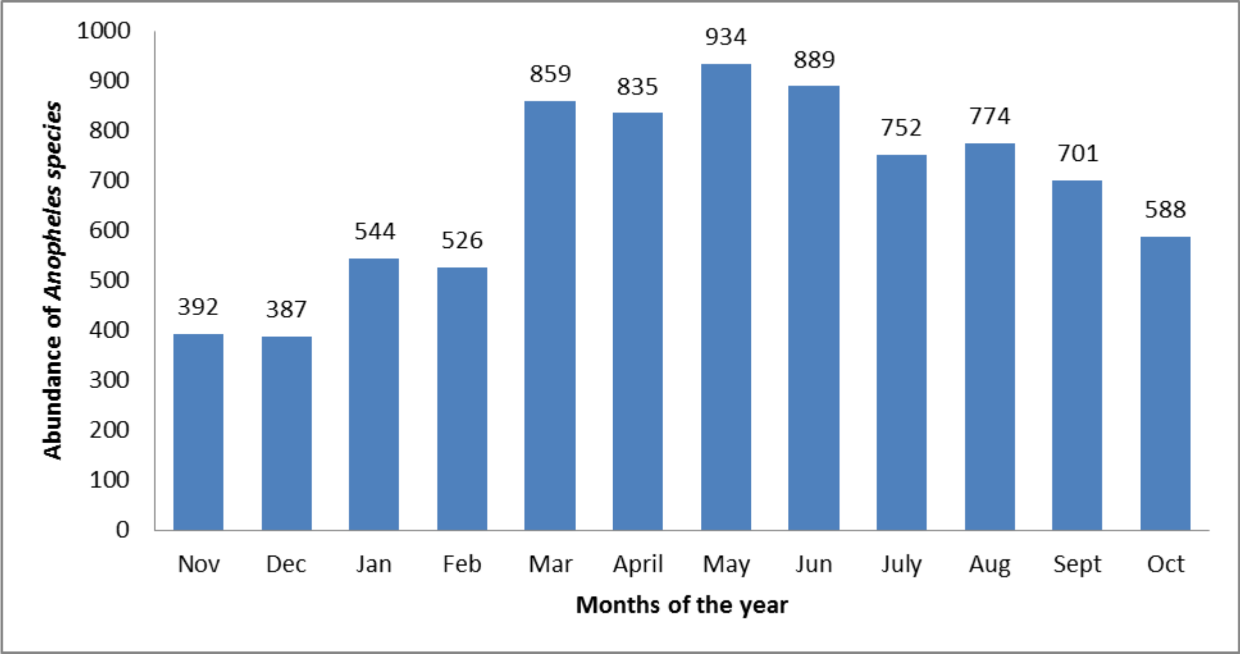


Figure 4a: Monthly abundance of *Anopheles* mosquitoes in the study area

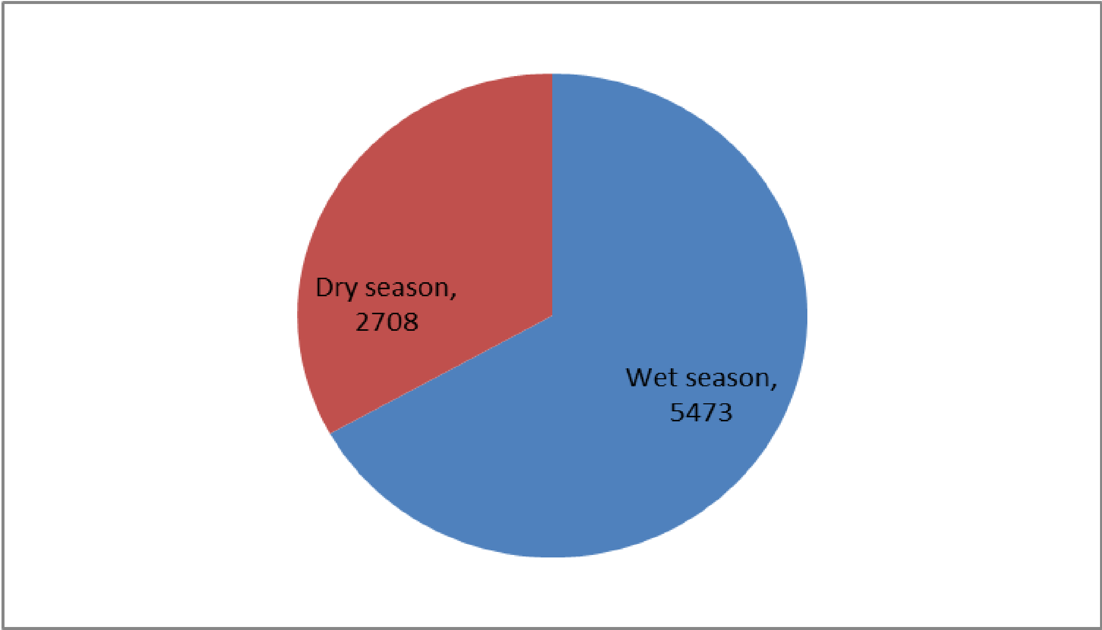


Figure 4b: Seasonal abundance of *Anopheles* mosquitoes in the study area

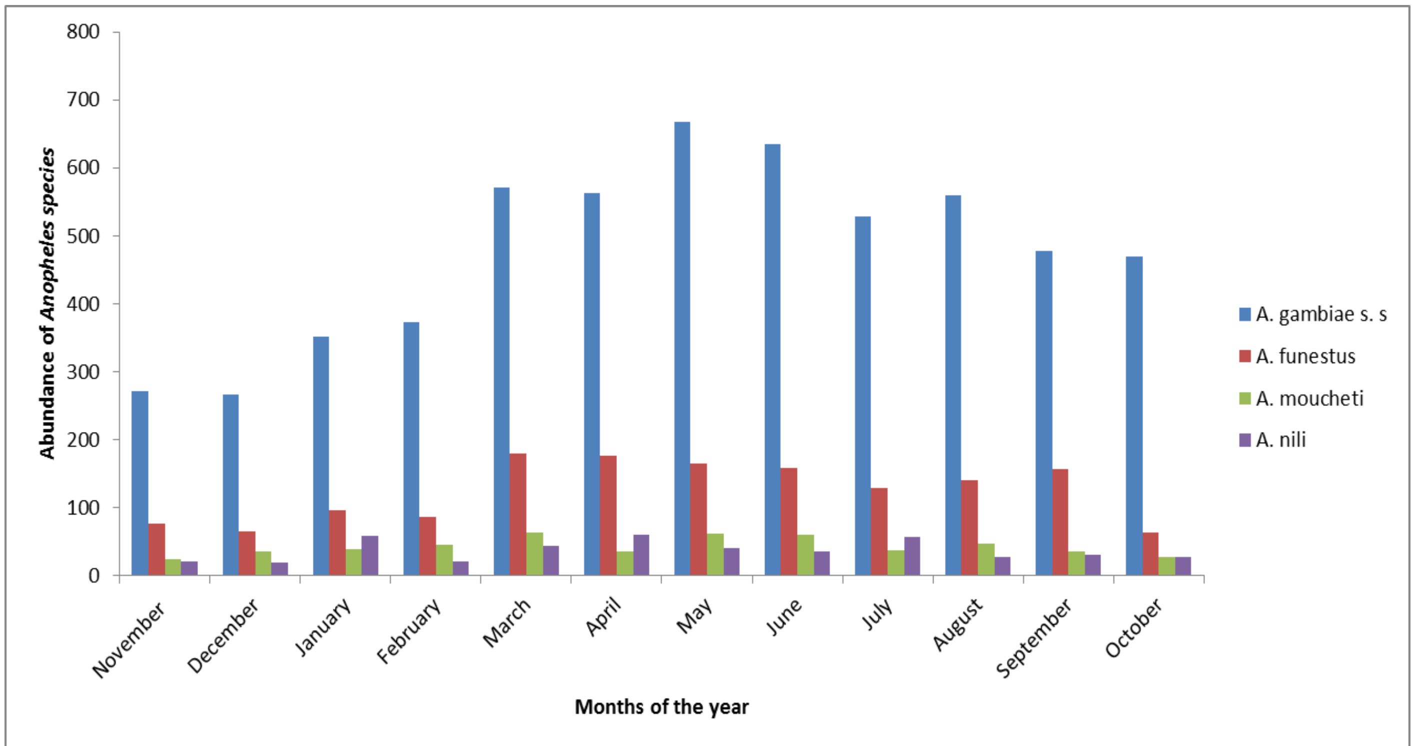


Figure 5a: Monthly abundance of different *Anopheles* species in the study area

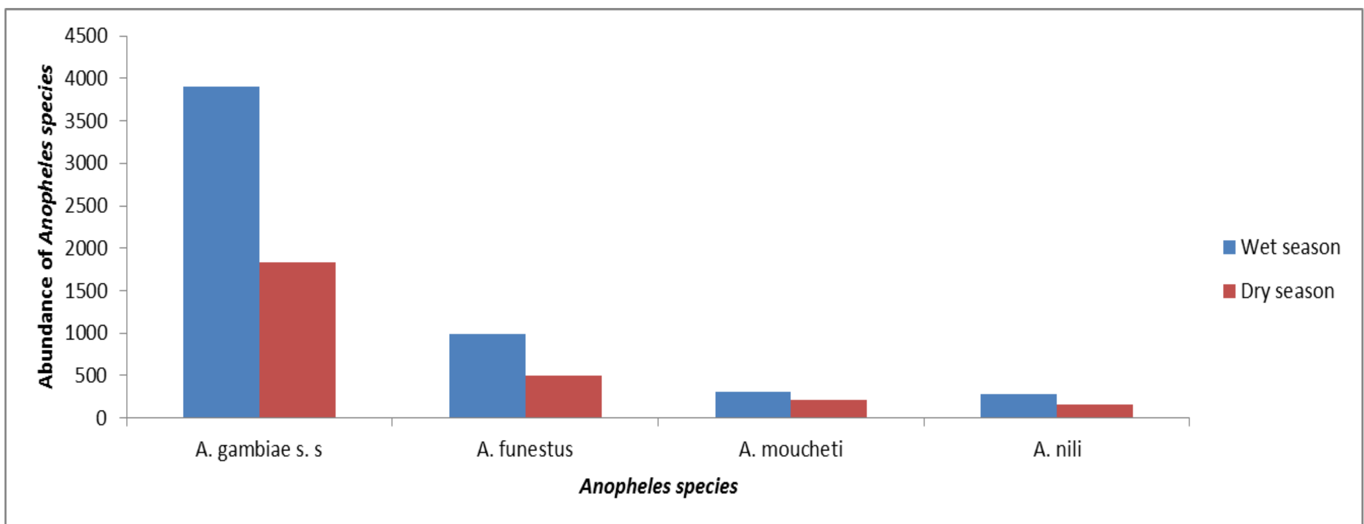


Figure 5b: Seasonal abundance of different *Anopheles* species in the study area

4.1.4 Climatic factors influencing the survival and population of *Anopheles* mosquitoes in the study area.

4.1.4.1 Temperature

The overall mean±se of temperature within the study area was 27.7ºC±0.4ºC. The mean±se abundance of the total *Anopheles* mosquitoes in the study area was 681.8±54.9 (Figure 6a). There was no significant correlation (r = -0.05; P = 0.878) between the total *Anopheles* mosquito abundance and temperature (Figure 6b). The mean±se abundance of the total *An. gambiae* s. s. in the study area was 477.8±38.9 (Figure 6a). There was no significant correlation (r = -0.12; P = 0.713) between the *An. gambiae* s. s. abundance and temperature (Figure 6b). The mean±se abundance of the total *An. funestus* in the study area was 124.6±12.8 (Figure 6a). There was no significant correlation (r = 0.08; P = 0.803) between the *An. funestus* abundance and temperature (Figure 6b). The mean±se abundance of total *An. moucheti* in the study area was 43.6±3.9 (Figure 6a). There was no significant correlation (r = 0.19; P = 0.558) between the *An. moucheti* abundance and temperature (Figure 6b). The mean±se abundance of the total *An. nili* in the study area was 36.8±4.4 (Figure 6a). There was no significant correlation (r = -0.01; P = 0.982) between the *An. nili* abundance and temperature (Figure 6b).

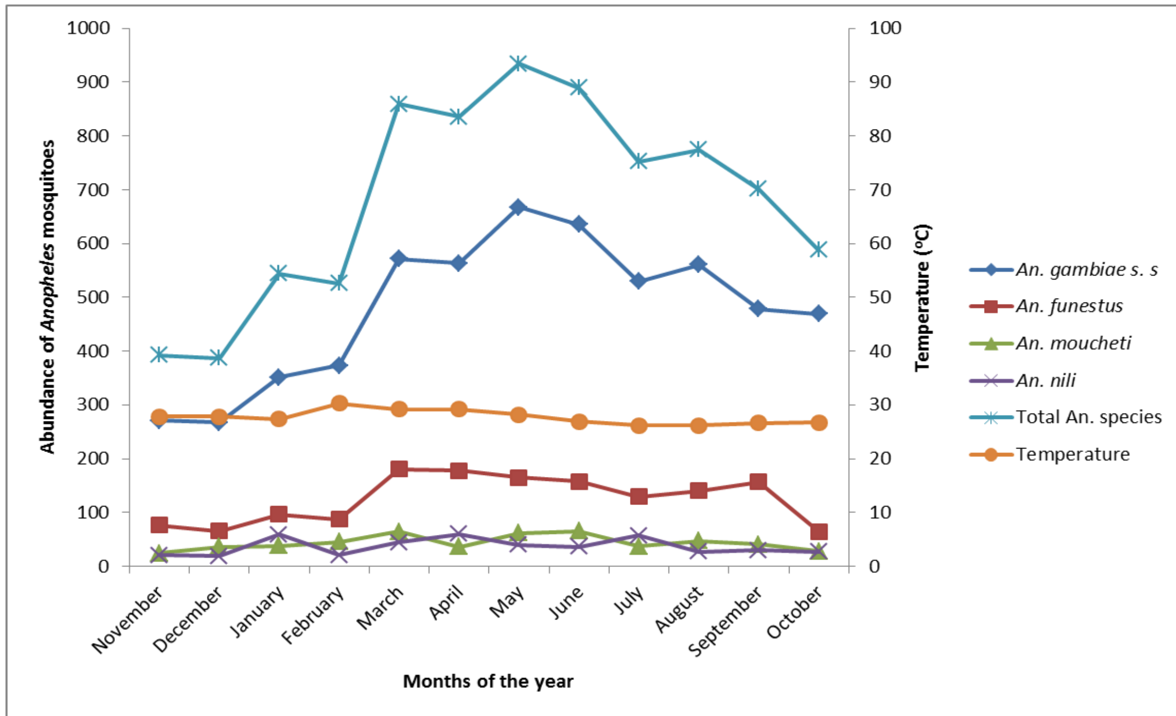


Figure 6a: Response of *Anopheles* mosquito populations to temperature

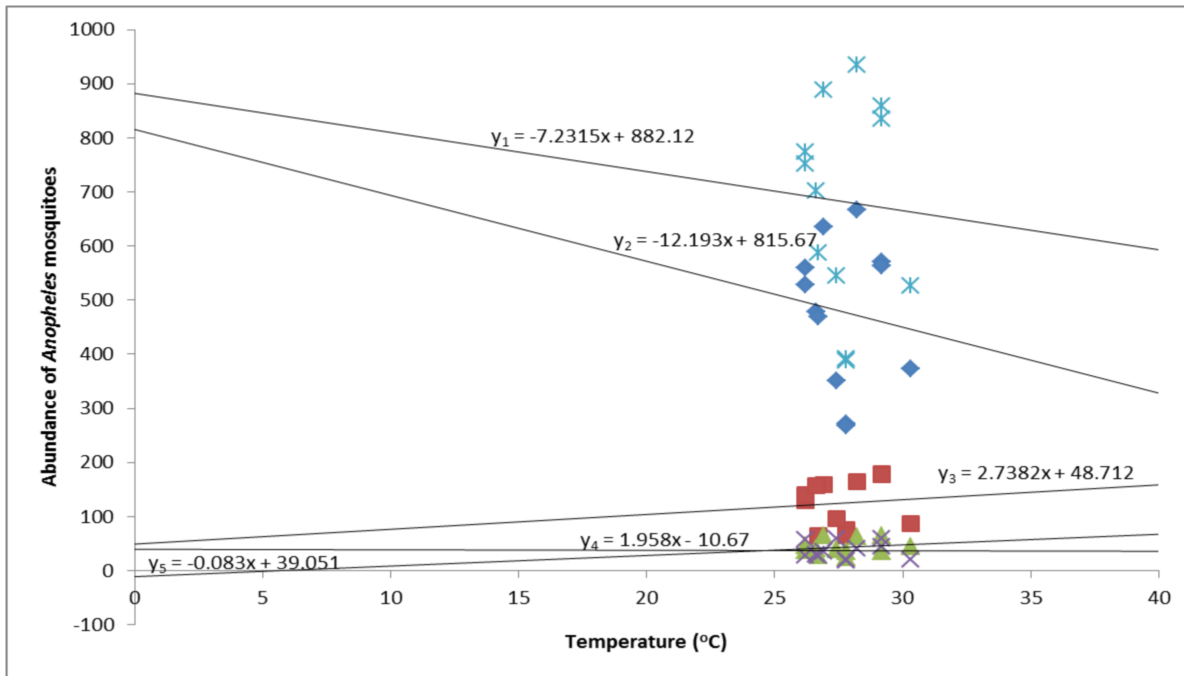


Figure 6b: Relationship between *Anopheles* mosquito populations and temperature
Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

4.1.4.2 Rainfall

The overall mean±se of rainfall within the study area was 291mm±79.0mm. The mean±se abundance of the total *Anopheles* mosquitoes in the study area was 681.8±54.9 (Figure 7a). There was significant strong positive correlation (r = 0.63; P = 0.029) between rainfall and *Anopheles* mosquito abundance (Figure 7b). The mean±se abundance of the total *An. gambiae* s. s. in the study area was 477.8±38.9 (Figure 7a). There was significant strong positive correlation (r = 0.66; P = 0.02) between rainfall and *An. gambiae* s. s. abundance (Figure 7b). The mean±se abundance of the total *An. funestus* in the study area was 124.6±12.8 (Figure 7a). There was no significant correlation (r = 0.56; P = 0.059) between the *An. funestus* abundance rainfall (Figure 7b). The mean±se abundance of total *An. moucheti* in the study area was 43.6±3.9 (Figure 7a). There was no significant correlation (r = 0.18; P = 0.0.575) between the *An. moucheti* abundance and rainfall (Figure 7b). The mean±se abundance of the total *An. nili* in the study area was 36.8±4.4 (Figure 7a). There was no significant correlation (r = 0.32; P = 0.306) between the *An. nili* abundance and rainfall (Figure 7b).

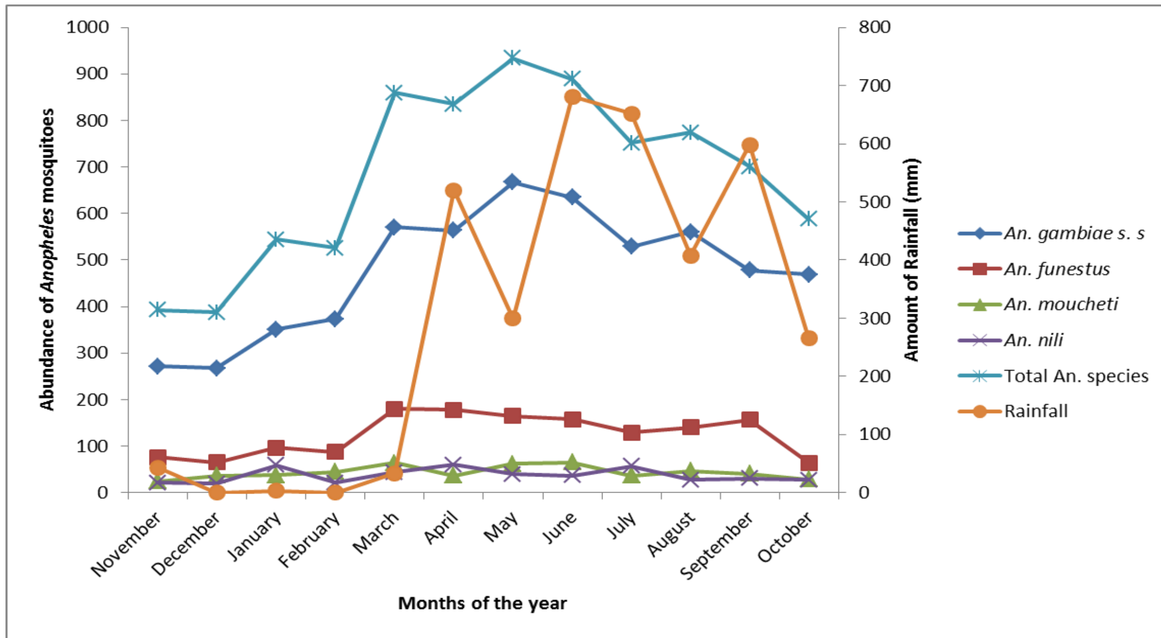


Figure 7a: Response of *Anopheles* mosquito populations to amount of rainfall

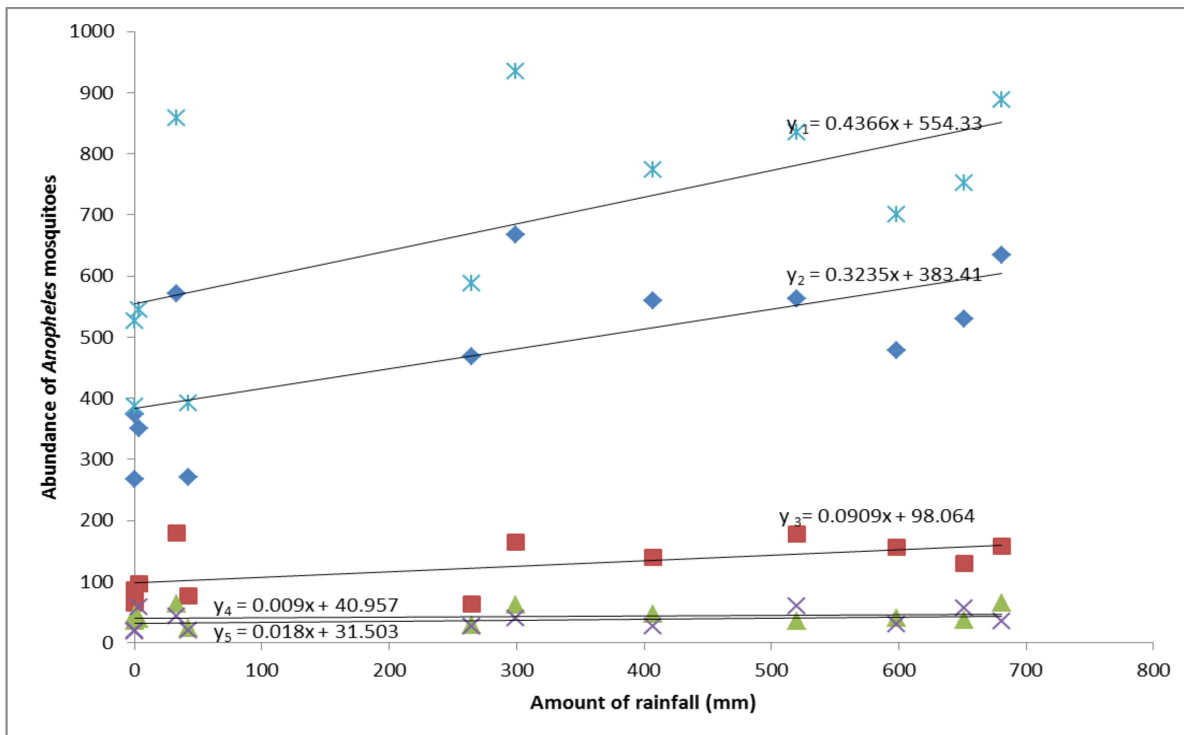


Figure 7b: Relationship between *Anopheles* mosquito populations and amount of rainfall
Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

4.1.4.3 Relative humidity

The overall mean±se of relative humidity within the study area was 79.3%±2.3%. The mean±se abundance of the total *Anopheles* mosquitoes in the study area was 681.8±54.9 (Figure 8a).There was no significant correlation (r = 0.47; P = 0.122) between the *Anopheles* species abundance and relative humidity (Figure 8b). The mean±se abundance of the total *An. gambiae* s. s. in the study area was 477.8±38.9 (Figure 8a). There was no significant correlation (r = 0.54; P = 0.07) between the *An. gambiae* s. s. abundance and relative humidity (Figure 8b). The mean±se abundance of the total *An. funestus* in the study area was 124.6±12.8 (Figure 8a). There was no significant correlation (r = 0.37; P = 0.231) between the *An. funestus* abundance and relative humidity (Figure 8b). The mean±se abundance of total *An. moucheti* in the study area was 43.6±3.9 (Figure 8a). There was no significant correlation (r = 0.02; P = 0.951) between the *An. moucheti* abundance and relative humidity (Figure 8b). The mean±se abundance of the total *An. nili* in the study area was 36.8±4.4 (Figure 8a). There was no significant correlation (r = 0.04; P = 0.899) between the *An. nili* abundance relative humidity (Figure 8b).

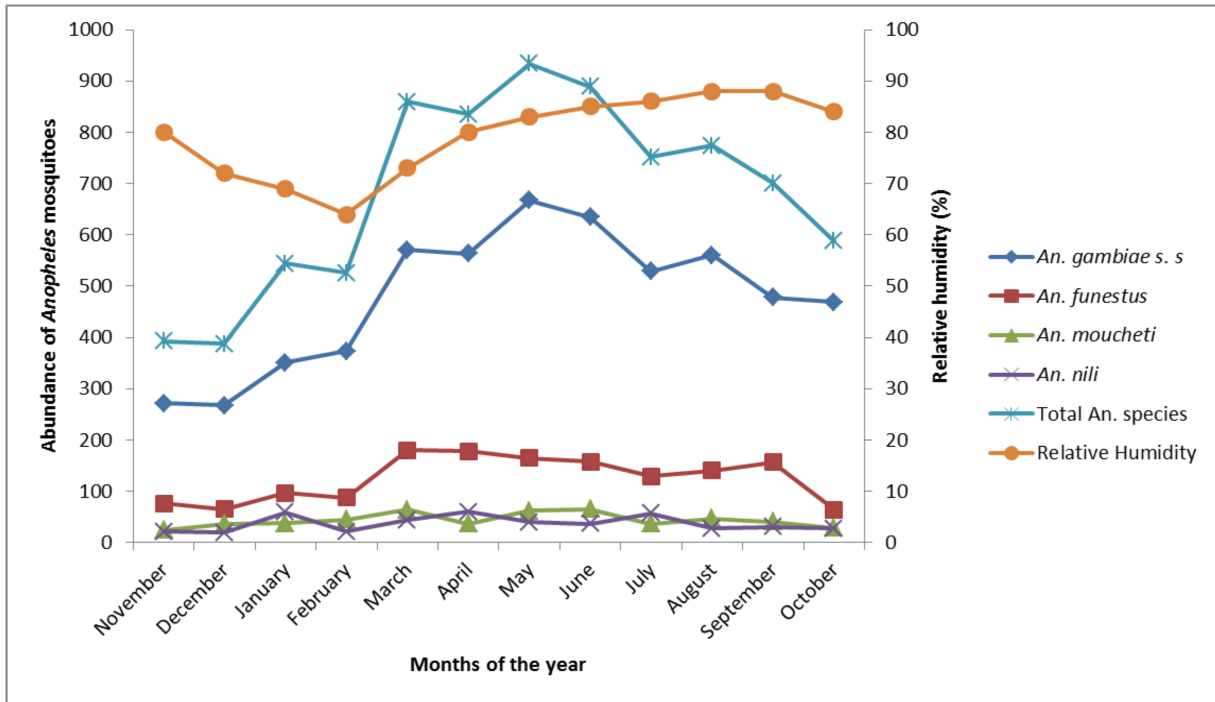


Figure 8a: Response of *Anopheles* mosquito populations to relative humidity

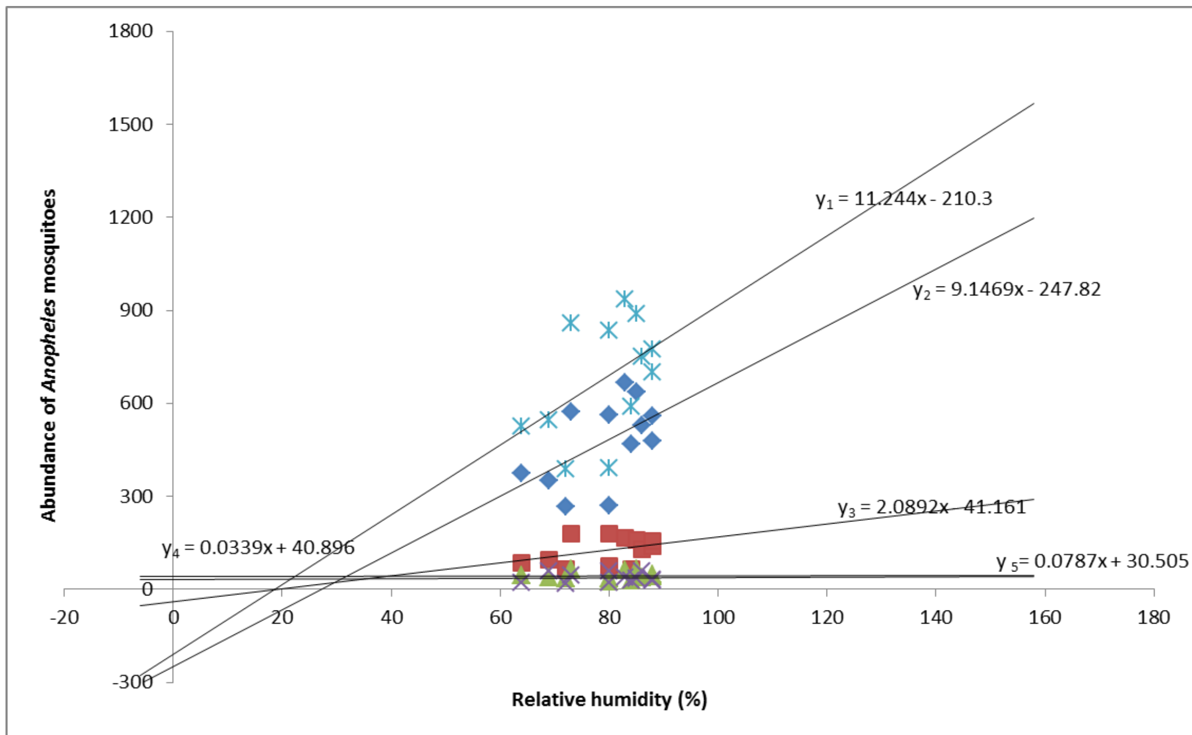


Figure 8b: Relationship between *Anopheles* mosquito populations and relative humidity
Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

4.2 Breeding ecology, physicochemical and biological factors operating in *Anopheles* mosquito habitats.

4.2.1 Breeding habitats of *Anopheles* mosquitoes in the study area

A total of 4127 *Anopheles* mosquito larvae were collected from nine different breeding habitats identified in the study area (Table 4). Both *An. gambiae* s. s. and *An. funestus* larvae were found in the 9 (100%) breeding habitats. *Anopheles moucheti* and *An. nili* larvae were found in 6 (66.7%) of the breeding habitats. A total of 2494 *An. gambiae* s. s. larvae were collected. The highest number of *An. gambiae* s. s. larvae, 549 (22.0%) was collected at the river banks. The least number, 9 (0.4%) was collected in head pans. There was significant difference in the distribution of *An. gambiae* s. s. larvae in various breeding habitats (P = 0.000; Appendix 18). A total of 1001 *An. funestus* larvae were collected. The highest number of *An. funestus* larvae, 259 (25.9%) was collected in river banks. The least number of *An. funestus* larvae, 9 (0.9%) was collected in head pans.There was significant difference in the distribution of *An. funestus* larvae in various breeding habitats (P = 0.000; Appendix 19). A total of 371 *An. moucheti* larvae were collected. The highest number of *An. moucheti* larvae, 205 (55.3%) was collected at the river bank. The least number of *An. moucheti* larvae, 4 (1.2%) was collected in drainage channels. There was significant difference in the distribution of *An. moucheti* larvae in various breeding habitats (P = 0.000; Appendix 20). A total of 261 *An. nili* larvae were collected. The highest number of *Anopheles nili* larvae, 94 (36.0%) was collected at the river banks. The least number of *An. nili* larvae, 2 (0.8%) were collected in clay pots and plastic drums / containers.There was significant difference in the distribution of *An. nili* larvae in various breeding habitats (P = 0.006; Appendix 21).

Table 4: *Anopheles* mosquito larvae distribution and abundance in different breeding habitats in Anambra East LGA, Anambra State.

Breeding habitat	*An. gambiae* s. s.		*An. funestus*		*An. moucheti*		*An. nili*		Total
	Abundance	Mean±SE	Abundance	Mean±SE	Abundance	Mean±SE	Abundance	Mean±SE	
Basin	103	8.6±2.3	11	0.9±0.9	-	-	-	-	114
Clay pots	273	22.8±4.6	81	6.8±1.6	7	0.6±0.4	2	0.2±0.2	363
Canoes	291	24.3±2.4	93	7.8±1.2	27	2.3±0.8	60	5.0±1.7	471
Drainage channels	403	33.6±6.9	149	12.4±3.1	4	0.3±0.2	30	2.5±1.6	586
Head pans	9	0.8±0.8	9	0.8±0.6	-	-	-	-	18
Plastic drum/ containers	399	33.3±4.3	143	11.9±3.4	9	0.8±0.6	2	0.2±0.2	553
Puddles	287	23.9±6.5	130	11.2±3.0	-	-	-	-	421
River banks	549	45.8±3.2	259	21.6±2.2	205	17.1±2.8	94	7.8±2.3	1107
Swamps	180	15.0±4.3	122	10.2±2.9	119	9.9±3.5	90	6.1±2.3	494
Total	2494	207.8±28.3	1001	83.4±11.4	371	30.9±3.1	261	21.8±3.8	4127

4.2.2 Monthly and seasonal availability of *Anopheles* mosquito breeding habitats.

The monthly and seasonal availability of *Anopheles* mosquito breeding habitats is shown in Table 5. Of the nine breeding habitats of *Anopheles* mosquito encountered in the study area, canoe, plastic drums/containers and river banks showed 100% monthly and seasonal availability. Basins showed 75% (9/12) monthly availability; 40% (2/5) availability in the dry season and 100% (7/7) availability in the wet season. Broken clay pots showed 91.7% (11/12) monthly availability; 80% (4/5) availability in the dry season and 100% (7/7) availability in the wet season. Drainage channels showed 83.3% (10/12) monthly availability; 60% (3/5) availability in the dry season and 100% (7/7) availability in the wet season. Head pans showed 16.7% (2/12) monthly availability; 20% (1/5) availability in the dry season and 14.3% (1/7) availability in the wet season. Puddles and swamps showed 66.7% (8/12) monthly availability; 40% (2/5) availability in the dry season and 85.7% (6/7) availability in the wet season.

Table 5: Monthly and seasonal availability of *Anopheles* mosquito breeding habitats

Breeding habitats	Dry season					Wet season						
	November	December	January	February	March	April	May	June	July	August	September	October
Basin	-	-	+	-	+	+	+	+	+	+	+	+
Broken clay pots	+	-	+	+	+	+	+	+	+	+	+	+
Canoes	+	+	+	+	+	+	+	+	+	+	+	+
Drainage channels	+	-	+	-	+	+	+	+	+	+	+	+
Head pans	-	-	-	-	+	+	-	-	-	-	-	-
Plastic drum/ containers	+	+	+	+	+	+	+	+	+	+	+	+
Puddles	-	-	+	-	+	+	+	+	+	+	+	-
River banks	+	+	+	+	+	+	+	+	+	+	+	+
Swamps	-	-	+	-	+	+	+	+	+	+	+	-

Key: + means available, - means not available.

4.2.3 *Anopheles* mosquito larval habitats in various communities.

A total of 8 *Anopheles* mosquito breeding habitats were encountered at Aguleri, 7 at Igbariam and 8 at Nsugbe (Table 6). The abundance of *Anopheles* mosquito larvae was 1341 (32.5%) at Aguleri, 1350 (32.7%) at Igbariam and 1436 (34.8%) at Nsugbe. In Aguleri, River bank produced the highest number of *Anopheles* mosquito larvae, 298 (22.2%) while head pans produced the least number of *Anopheles* mosquito larvae, 18 (1.3%). In Igbariam, River bank produced the highest number of *Anopheles* mosquito larvae, 331 (24.5%) while broken clay pots produced the least number of *Anopheles* mosquito larvae, 121 (9.0%). In Nsugbe, River bank produced the highest number of *Anopheles* mosquito larvae, 478 (33.3%) while basin produced the least number of *Anopheles* mosquito larvae, 114 (7.9%).

Table 6: Distribution of *Anopheles* mosquito larvae in their breeding sites within the communities studied

Breeding habitat	Communities studied			Total
	Aguleri	**Igbariam**	**Nsugbe**	
Basin	-	-	114	114
Broken clay pot	115	121	127	363
Canoe	159	160	152	471
Drainage channel	250	211	125	586
Head pans	18	-	-	18
Plastic drum/ container	199	194	160	553
Puddle	105	160	156	421
River banks	298	331	478	1107
Swamp	197	173	124	494
Total	**1341**	**1350**	**1436**	**4127**

4.2.4 Monthly distribution and abundance of *Anopheles* mosquito larvae

The monthly distribution and abundance of the *Anopheles* mosquito larvae is shown in Table 7. The monthly mean abundance of *An. gambiae* s. s. larvae was 207.8±25.6**.** The highest number, 342 (13.7%) of *An. gambiae* s. s. larvae was collected in the month of June while the least number, 89 (3.6%) was collected in the month of December. The monthly mean abundance of *An. funestus* larvae was 83.4±11.4**.** The highest number, 143 (14.3%) of *An. funestus* larvae was collected in the month of March while the least number, 25 (2.5%) was collected in the month of October. The monthly mean abundance of *An. moucheti* larvae was 30.9±3.1**.** The highest number, 49 (13.2%) of *An. moucheti* larvae was collected in the month of March while the least number, 15 (4.0%) was collected in the month of November. The monthly mean abundance of *An. nili* larvae was 21.8±3.8**.** The highest number, 42 (16.1%) of *An. nili* larvae was collected in the month of January while the least number, 5 (1.9%) was collected in the month of February. There was significant difference in the monthly abundance of the different *Anopheles* species larvae collected (P = 0.000; Appendix 22)

Table 7: Monthly distribution and abundance of *Anopheles* mosquito larvae

Month	***An. gambiae* s. s.**	***An. funestus***	***An. mouchetti***	***An. nili***	**Total**
November	117	45	15	12	189
December	89	30	21	9	149
January	113	57	28	42	240
February	117	59	34	4	214
March	273	143	49	36	501
April	259	129	21	40	449
May	225	106	45	19	395
June	342	108	45	21	516
July	266	82	31	32	411
August	301	102	34	18	455
September	264	115	26	23	428
October	128	25	22	5	180
Total	**2494**	**1001**	**371**	**261**	**4127**
mean±se	**207.8±25.6**	**83.4±11.4**	**30.9±3.1**	**21.8±3.8**	**343.9±39.7**

4.2.5 Seasonal distribution and abundance of *Anopheles* mosquito larvae

The seasonal distribution and abundance of the *Anopheles* mosquito larvae is shown in Table 8. A total of 2834 (68.7%) *Anopheles* mosquito larvae were collected in the wet season whereas 1293 (31.3%) larvae were collected in the dry season. There was no significant difference between the dry and wet season abundance of *Anopheles* mosquito larvae collected from the study (P = 0.066; Appendix 23). *An. gambiae* s. s. larvae recorded the highest abundance level of 54.8% (709/1293) and 63.0% (1785/2834) for dry and wet seasons respectively. *An. nili* larvae recorded the lowest abundance level of 8.0% (103/1293) and 5.6% (158/2834) for dry and wet seasons respectively. There was significant difference in the abundance of different *Anopheles* species larvae collected in dry season (P = 0.002; Appendix 24) and also in the wet season (P = 0.000; Appendix 25). All the *Anopheles* species collected were more abundant in the wet season than in the dry season with the abundance level of 71.6% (1785/2494) for *An. gambiae* s. s., 66.6% (667/1001) for *An. funestus*, 60.4% (224/371) for *An. moucheti* and 60.5% (158/261) for *An. nili*. There was significant difference between the dry and wet season abundance of *An. gambiae* s. s. larvae (P = 0.02; Appendix 26). There was no significant difference between the dry and wet season abundance of *An. funestus* larvae. (P = 0.234; Appendix 27), *An. moucheti* larvae (P = 0.704; Appendix 28) and *An. nili* larvae (P = 0.348; Appendix 29).

Table 8: Seasonal distribution and abundance of *Anopheles* mosquito larvae from the study area.

Anopheles species	Dry season		Wet season		Total
	Abundance (%)	Mean±SE	Abundance (%)	Mean±SE	
An. gambiae s. s	709 (54.8)	141.8±33.2	1785 (63.0)	255.0±25.3	2494
An. funestus	334 (25.8)	66.8±19.7	667 (23.5)	95.3±12.9	1001
An. moucheti	147 (11.4)	29.4±5.9	224 (7.9)	32.0±3.8	371
An. nili.	103 (8.0)	20.6±7.7	158 (5.6)	22.6±4.2	261
Total	**1293 (31.3)**	**258.6±62.4**	**2834 (68.7)**	**404.9±40.3**	**4127**

4.2.6 Monthly productivity of *Anopheles* mosquito breeding habitats

The monthly Productivity of *Anopheles* mosquito larval habitats is shown in Table 9. In overall, the most productive habitat was river banks with *Anopheles* mosquito larval abundance of 1107 (26.8%). The least productive habitat was head pans with *Anopheles* mosquito larval abundance of 18 (0.4%). In the months of October, November, December, January, February, March, April, May, June, July and September, river bank was the most productive habitat with 28.9% (52/180), 36.6% (71/189), 63.1% (94/149), 39.2% (94/240), 47.2% (101/214), 21.8% (109/501), 21.6% (97/449), 23.3% (92/395), 21.5% (111/516), 29.0% (119/411) and 24.5% (105/428) abundance levels of *Anopheles* mosquitoes recorded respectively. In the months of August, swamp was the most productive habitat with 22.4% (102/455) abundance levels of *Anopheles* mosquitoes reported. There was significant difference in the monthly productivity of *Anopheles* mosquito breeding habitat (P = 0.000; Appendix 30).

Table 9: Monthly Productivity of *Anopheles* mosquito larval habitats

Breeding habitat	**Abundance of *Anopheles species* Larvae**												
	Oct	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Total**
Basin	8	0	0	14	0	6	11	24	18	11	13	9	114
Clay pot	13	28	0	20	22	50	65	19	45	40	37	24	363
Canoes	42	49	38	50	57	43	43	26	38	24	32	29	471
Drainage channel	29	18	0	6	0	86	66	58	80	77	99	67	586
Head pan	0	0	0	0	0	7	11	0	0	0	0	0	18
Plastic drum/containers	36	23	17	21	34	85	64	55	89	43	35	51	553
Puddle	0	0	0	20	0	46	57	52	68	34	75	69	421
River banks	52	71	94	94	101	109	97	92	111	119	62	105	1107
Swamp	0	0	0	15	0	69	35	69	67	85	102	74	494
TOTAL	**180**	**189**	**149**	**240**	**214**	**501**	**449**	**395**	**516**	**411**	**455**	**428**	**4127**

4.2.6.1 Monthly productivity of *An. gambiae* s. s. breeding habitats

The Productivity of *An. gambiae* s. s. breeding habitats is shown in Table 10. The most productive habitat for *An. gambiae* s. s. was river bank with abundance level of 22.0% (549/2494). In the months of November, December, January, February, July, September and October, river bank was was the most productive habitat with 25.6% (30/117), 53.9% (48/89), 35.4% (40/113), 41.0% (48/117), 23.7% (63/266), 22.3% (59/264) and 25.0% (32/128) abundance levels of *An. gambiae* s. s. respectively. In the months of, March, plastic drum/container was the most productive habitat with 22.0% (60/273) abundance level of *An. gambiae* s. s. respectively. In the months of, May, June, and August, drainage channel was the most productive habitat with 16.0% (36/225), 17.3% (59/342), and 21.9% (66/301) abundance levels of *An. gambiae* s. s. respectively. In the month of April, clay pot was the most productive habitat with 19.7% (51/259) abundance level of *An. gambiae* s. s. There was significant difference in the monthly productivity of *An. gambiae* s. s breeding habitats (P = 0.000; Appendix 31).

Table 10: Monthly Productivity of *An. gambiae* s. s. breeding habitats

Breeding habitats	**Abundance of *An. gambiae* s. s. Larvae**												**Total**
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	
Basin	0	0	14	0	6	0	24	18	11	13	9	8	103
Clay pot	18	0	9	8	38	51	17	36	36	37	10	13	273
Canoe	29	24	16	42	28	21	22	35	14	18	16	26	291
Drainage channel	17	0	6	0	49	35	36	59	59	66	53	23	403
Head pans	0	0	0	0	0	9	0	0	0	0	0	0	9
Plastic drum/ container	23	17	13	19	60	35	45	53	42	26	40	26	399
Puddle	0	0	0	0	32	41	35	58	30	51	40	0	287
River bank	30	48	40	48	49	49	30	56	63	45	59	32	549
Swamp	0	0	15	0	11	18	16	27	11	45	37	0	180
TOTAL	**117**	**89**	**113**	**117**	**273**	**259**	**225**	**342**	**266**	**301**	**264**	**128**	**2494**

4.2.6.2 Monthly productivity of *An. funestus* breeding habitats

The Productivity of *An. funestus* breeding habitats is shown in Table 11. River bank was also the most productive habitat for *An. funestus* with reported abundance level of 25.9% (259/1001). In the months of November, December, February, May, July, and October, river bank was the most productive habitat with 57.8% (26/45), 53.3% (16/30), 45.6% (26/57), 32.1% (34/106), 40.2% (33/82) and 40.0% (10/25) abundance levels of *An. funestus* respectively. In the month of March, Swamp was the most productive habitat with abundance levels of 20.3% (29/143). In the month of August, drainage channel was the most productive habitat with 30.4% (31/102) abundance levels of *An. funestus*. In the months of January and September, puddle was the most productive habitat with 35.1% (20/57) and 25.2% (29/115) abundance levels of *An. funestus* respectively. In the months of April and June, Plastic drum/ container was the most productive habitat with 22.5% (29/129) and 31.5% (34/108) abundance levels of *An. funestus* respectively. There was significant difference in the monthly productivity of *An. funestus* breeding habitat (P = 0.006; Appendix 32).

Table 11: Monthly Productivity of *An. funestus* breeding habitats

Breeding habitats	Abundance of *An. funestus* Larvae												Total
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	
Basin	0	0	0	0	0	11	0	0	0	0	0	0	11
Clay pot	10	0	6	14	12	14	2	9	2	0	12	0	81
Canoe	8	14	10	4	5	12	3	2	6	10	13	6	93
Drainage channel	1	0	0	0	25	14	22	18	18	31	14	6	149
Head pan	0	0	0	0	7	2	0	0	0	0	0	0	9
Plastic drum /container	0	0	8	15	25	29	8	34	1	9	11	3	143
Puddle	0	0	20	0	14	16	17	10	4	24	29	0	130
River bank	26	16	13	26	26	14	34	21	33	17	23	10	259
Swamp	0	0	0	0	29	17	20	14	18	11	13	0	122
TOTAL	**45**	**30**	**57**	**59**	**143**	**129**	**106**	**108**	**82**	**102**	**115**	**25**	**1001**

4.2.6.3 Monthly productivity of *An. moucheti* breeding habitats

The Productivity of *An. moucheti* breeding habitats is shown in Table 12. River bank was the most productive habitat for *An. moucheti* that recorded abundance level of 55.3% (205/371). In the months of November, December, January, February, March, April and June and October, river bank was the most productive habitat with abundance levels of 100% (15/15), 100% (21/21), 60.7% (17/28), 76.5% (26/34), 69.4% (34/49), 100% (21/21), 64.4% (29/45) and 45.5% (10/22) respectively. In the months of May, July, August and September, swamp was the most productive habitat with 73.3% (33/45), 45.2% (14/31), 85.3% (29/34) and 50.0% (17/34) abundance levels respectively. There was no significant difference in the monthly productivity of *An. moucheti* breeding habitats (P = 0.655; Appendix 33).

Table 12: Monthly Productivity of *An. moucheti* breeding habitats.

Breeding habitat	Abundance of *An. moucheti* Larvae												Total
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	
Clay pot	0	0	5	0	0	0	0	0	0	0	2	0	7
Canoe	0	0	6	8	0	0	0	1	4	3	0	5	27
Drainage channel	0	0	0	0	0	0	0	2	0	2	0	0	4
Plastic drum / containers	0	0	0	0	0	0	0	2	0	0	0	7	9
River Banks	15	21	17	26	34	21	12	29	13	0	7	10	205
Swamp	0	0	0	0	15	0	33	11	14	29	17	0	119
TOTAL	**15**	**21**	**28**	**34**	**49**	**21**	**45**	**45**	**31**	**34**	**26**	**22**	**371**

4.2.6.4 Monthly productivity of *An. nili* breeding habitats

The Productivity of *An. nili* breeding habitats is shown in Table 13. River bank was the most productive habitat for *An. nili* that recorded abundance level of 36.0% (94/261). In the months of October, November, and February, Canoe was the most productive habitat with abundance levels of 100% (5/5), 100% (12/12), and 75.0% (3/4) respectively. In the months of March, June, July and August, swamp was the most productive habitat with 38.9% (14/36), 71.4% (15/21), 62.5% (20/32) and 94.4% (17/18) abundance levels respectively. In the months of December, January, May and Sepetember, river bank was the most productive habitat with abundance level of 100% (9/9), 57.1% (24/42), 84.2% (16/19) and 69.6% (16/23). In the month of April, drainage channel was the most productive habitat with abundance level of 42.5% (17/40). There was no significant difference in the monthly productivity of *An. nili* breeding habitats (P = 0.748; Appendix 34).

Table 13: Monthly Productivity of *An. nili* larval habitat

Breeding habitat	Abundance of *An. nili* Larvae												Total
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	
Clay pot	0	0	0	0	0	0	0	0	2	0	0	0	2
Canoe	12	0	18	3	10	10	1	0	0	1	0	5	60
Drainage channel	0	0	0	0	12	17	0	1	0	0	0	0	30
Plastic drum / container	0	0	0	0	0	0	2	0	0	0	0	0	2
River bank	0	9	24	1	0	13	16	5	10	0	16	0	94
Swamp	0	0	0	0	14	0	0	15	20	17	7	0	90
Total	**12**	**9**	**42**	**4**	**36**	**40**	**19**	**21**	**32**	**18**	**23**	**5**	**261**

4.2.7 Biological characteristics of the breeding habitats.

The biological characteristics of the different *Anopheles* mosquito breeding habitats encountered in the study area are shown in Tables 14, 15 and 16 below. Invertebrate organisms (earthworm and other mosquito larvae), vertebrate organisms (fish and tadpole), algae and vegetations (aquatic plants, grasses and leaves from trees) were identified in the breeding habitats (Table 14). Six different bacterial isolates: *Pseudomonas* species*, Bacillius* species*, Serratia* species*, Micrococcus* species *and Eschericia coli* were found in the breeding habitats (Table 15). Also, fungal isolates: *Candida* species, *Emmonsia* species, *Chrysonilia* species, *Aspergillus* species, *Trichosporium* species and *Scedosporium* species were identified in the breeding habitats (Table 16). Only *Salmonella* species and other mosquito larvae were found in all the breeding habitats.

Table 14: Biological characteristics of *Anopheles* mosquito breeding sites (Flora and Fauna)

Breeding habitats	Flora			Invertebrate fauna						Vertebrate Fauna	
	Algae	Emergent vegetation	Aquatic vegetation	*Culex quinquefasciatus*	*Aedes albopictus*	*Aedes aegypti*	*Mansonia uniformis*	*Mansonia africana*	Earthworms	Tadpoles / toads	Fish
Basin	-	-	-	+	+	+	+	+	-	-	-
Broken clay pots	-	-	-	+	+	+	+	+	+	-	-
Canoes	-	-	-	+	+	+	+	+	-	-	-
Drainage channels	+	+	+	+	+	+	+	+	+	-	-
Head pans	-	-	-	+	+	+	+	+	-	-	-
Plastic drum / containers	-	-	-	+	+	+	+	+	-	-	-
Puddles	+	+	+	+	+	+	+	+	-	-	-
River bank		+	+	+	+	+	+	+	-	+	+
Swamps	+	+	+	+	+	+	+	+	+	+	-

Key: + means Present, - means Absent

Table 15: Biological characteristics of *Anopheles* mosquito breeding sites (Bacterial isolates)

Breeding habitats	*Pseudomonas* species	*Bacillius* species	*Serratia* species	*Micrococcus* species	*Salmonella* species	*Escherecia coli*
Basin	+	-	-	-	+	+
Broken clay pots	-	-	-	+	+	-
Canoes	-	-	-	-	+	+
Drainage channels	+	-	-	+	+	+
Head pans	-	-	+	-	+	-
Plastic drum / container	+	+	-	+	+	+
Puddles	-	+	-	+	+	+
River bank	-	-	-	-	+	+
Swamps	-	-	-	+	+	-

Key: + means Present, - means Absent

Table 16: Biological characteristics of *Anopheles* mosquito breeding sites (Fungal isolates)

Breeding habitats	***Candida* spp**	***Emmonsia* spp**	***Chrysonilia* spp**	***Aspergillus* spp**	***Trichosporium* spp**	***Scedosporium* spp**
Basin	+	-	-	-	-	-
Broken clay pots	-	-	-	+	-	-
Canoes	+	-	-	+	-	-
Drainage channels	+	-	+	+	+	+
Head pans	-	-	-	-	-	-
Plastic drum/ containers	+	-	-	-	-	-
Puddles	+	-	-	+	+	+
River bank	+	+	+	+	-	-
Swamps	-	+	+	+	-	-

Key: + means Present, - means Absent

4.2.8 Physico-chemical characteristics of the breeding habitats

The mean physical and chemical characteristics of the breeding habitats of *Anopheles* mosquitoes are shown in the Table 17 below. The parameters tested include: temperature, pH, Biochemical Oxygen Demand (BOD), Total Dissolved Solute (TDS), Total Soluble Solute (TSS), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), Salinity, Sulphur concentration (S), Iron concentration (Fe), Cadmium concentration (Cd), Lead concentration (Pb), Manganese concentration (Mn) and Phosphorous concentration (P).

Table 17: Physico-Chemical characteristics of *Anopheles species* breeding habitats.

Breeding sites	T	pH	BOD	TDS	TSS	COD	DO	Salinity	S	Fe	Cd	Pb	Mn	P
Basin	28.6	7.16	90.52	71.1	201	21.54	6.87	0.11	40.4	1.6	0.12	-	0.16	2.0
Broken clay pots	26.8	7.27	98.57	70.6	522	29.97	6.69	0.03	43.2	1.6	0.02	-	0.36	2.3
Canoes	28.1	7.22	24.54	561.9	411.6	33.2	5.20	0.13	51.2	1.33	0.01	1.17	0.29	1.48
Drainage channels	30.2	7.68	52	1136	290	51.98	6.56	0.03	17.28	2.23	0.11	0.3	0.45	2.72
Head pans	29.2	7.23	57.1	521.6	253	22.91	4.70	0.10	50.6	0.65	0.02	1.72	0.15	1.42
Plastic drum / containers	29.1	7.13	23.7	145.8	131.3	29.4	4.3	0.04	32.8	1.1	0.13	-	0.11	0.24
Puddles	27.3	7.34	25.25	133.7	310	29.98	5.27	0.03	21.9	1.96	0.01	-	0.2	0.2
River bank	27.3	7.4	18.89	163	125	86.95	1.91	0.01	37.41	0.35	0.02	-	0.13	1.50
Swamps	27.5	6.77	21.96	212	190	15.97	5.99	0.05	24	0.76	0.03	1.8	0.18	1.75

4.2.8.1 Relationship between *Anopheles* mosquitoes and the physicochemical parameters of their breeding habitats.

The relationships between *Anopheles* mosquito larvae abundance and the physicochemical parameters of their breeding habitats are shown in Figures 9 to 22.

There was no significant correlation between the water temperature and *An. gambiae* s. s. larvae (r = - 0.11, P = 0.784), *An. funestus* larvae (r = - 0.22, P = 0.567), *An. moucheti* larvae (r = - 0.43, P = 0.18), *An. nili* larvae (r = - 0.29, P = 0.451) and total *Anopheles* mosquito larvae (r = - 0.25, P = 0.526). (Figure 9)

There was no significant correlation between the water pH and *An. gambiae* s. s. larvae (r = 0.47, P = 0.207), *An. funestus* larvae (r = 0.29, P = 0.457) *An. moucheti* larvae (r = - 0.17, P = 0.655) *An. nili* larvae (r = - 0.21, P = 0.580) and total *Anopheles* mosquito larvae (r = 0.26, P = 0.495). (Figure 10)

There was no significant correlation between the Biochemical Oxygen Demand (BOD) and *An. gambiae* s. s. larvae (r = - 0.45, P = 0.219), *An. funestus* larvae (r = - 0.64, P = 0.066), *An. moucheti* larvae (r = - 0.49, P = 0.180), *An. nili* larvae (r = - 0.58, P = 0.103) and total *Anopheles* mosquito larvae (r = - 0.58, P = 0.101). (Figure 11)

There was significant strong positive correlation between the Chemical Oxygen Demand (COD) and *An. gambiae* s. s. larvae (r = 0.81, P = 0.009), *An. funestus* larvae (r = 0.80, P = 0.009) and total *Anopheles* mosquito larvae (r = 0.84, P = 0.004). However, There was no significant correlation between the Chemical Oxygen Demand (COD) and *An. moucheti* larvae (r = 0.64, P = 0.063), and *An. nili* larvae (r = 0.48, P = 0.191) (Figure 12)

There was significant strong negative correlation between the Dissolved Oxygen (DO) and *An. moucheti* larvae (r = - 0.67, P = 0.049). However, there was no significant correlation between the Dissolved Oxygen (DO) and *An. gambiae* s. s. larvae (r = - 0.52, P = 0.148), *An. funestus* larvae (r = - 0.65, P = 0.059), *An. nili* larvae (r = - 0.44, P = 0.240) and total *Anopheles* mosquito larvae (r = - 0.65, P = 0.057). (Figure 13)

There was no significant correlation between the Total Dissolved Solute (TDS) and *An. gambiae* s. s. larvae (r = 0.09, P = 0.820), *An. funestus* larvae (r = 0.02, P = 0.953), *An. moucheti* larvae (r = - 0.22, P = 0.574), *An. nili* larvae (r = 0.08, P = 0.830) and total *Anopheles* mosquito larvae (r = 0.12, P = 0.970). (Figure 14)

There was no significant correlation between the Total Dissolved Solute (TSS) and *An. gambiae* s. s. larvae (r = - 0.16, P = 0.675), *An. funestus* larvae (r = - 0.34, P = 0.379), *An. moucheti* larvae (r = - 0. 46, P = 0.210), *An. nili* larvae (r = - 0. 28, P = 0.471) and total *Anopheles* mosquito larvae (r = - 0.31, P = 0.421). (Figure 15)

There was significant strong negative correlation between the salinity and *An. funestus* larvae (r = - 0.74, P = 0.02). However, there was no significant correlation between the salinity and *An. gambiae* s. s. larvae (r = - 0.66, P = 0.051), *An. moucheti* larvae (r = - 0.39, P = 0.300), *An. nili* larvae (r = - 0.15, P = 0.696) and total *Anopheles* mosquito larvae (r = - 0.64, P = 0.061). (Figure 16)

There was no significant correlation between the sulphur concentration and *An. gambiae* s. s. larvae (r = - 0.36, P = 0.342), *An. funestus* larvae (r = - 0.46, P = 0.218), *An. moucheti* larvae (r = - 0.08, P = 0.839), *An. nili* larvae (r = - 0.11, P = 0.782) and total *Anopheles* mosquito larvae (r = - 0.33, P = 0.385). (Figure 17)

There was significant strong negative correlation between the iron concentration and *An. moucheti* larvae (r = - 0.69, P = 0.04). However, there was no significant correlation between the iron concentration and *An. gambiae* s. s. larvae (r = 0.02, P = 0.964), *An. funestus* larvae (r = - 0.21, P = 0.596), *An. nili* larvae (r = - 0.52, P = 0.152) and total *Anopheles* mosquito larvae (r = - 0.26, P = 0.498). (Figure 18)

There was no significant correlation between the cadmium concentration and *An. gambiae* s. s. larvae (r = 0.09, P = 0.817), *An. funestus* larvae (r = - 0.09, P = 0.813), *An. moucheti* larvae (r = - 0.33, P = 0.392), *An. nili* larvae (r = - 0.35, P = 0.359) and total *Anopheles* mosquito larvae (r = - 0.09, P = 0.810). (Figure 19)

There was no significant correlation between the lead concentration and *An. gambiae* s. s. larvae (r = - 0.57, P = 0.113), *An. funestus* larvae (r = - 0.34, P = 0.364), *An. moucheti* larvae (r = 0.09,

P = 0.815), *An. nili* larvae (r = 0.35, P = 0.360) and total *Anopheles* mosquito larvae (r = - 0.33, P = 0.385). (Figure 20)

There was no significant correlation between the manganese concentration and *An. gambiae* s. s. larvae (r = 0.16, P = 0.682), *An. funestus* larvae (r = - 0.02, P = 0.965), *An. moucheti* larvae (r = - 0.34, P = 0.378), *An. nili* larvae (r = - 0.07, P = 0.867) and total *Anopheles* mosquito larvae (r = - 0.004, P = 0.992). (Figure 21)

There was no significant correlation between the phosphorous concentration and *An. gambiae* s. s. larvae (r = - 0.10, P = 0.807), *An. funestus* larvae (r = - 0.16, P = 0.685), *An. moucheti* larvae (r = 0.05, P = 0.901), *An. nili* larvae (r = 0.20, P = 0.610) and total *Anopheles* mosquito larvae (r = - 0.06, P = 0.884). (Figure 22)

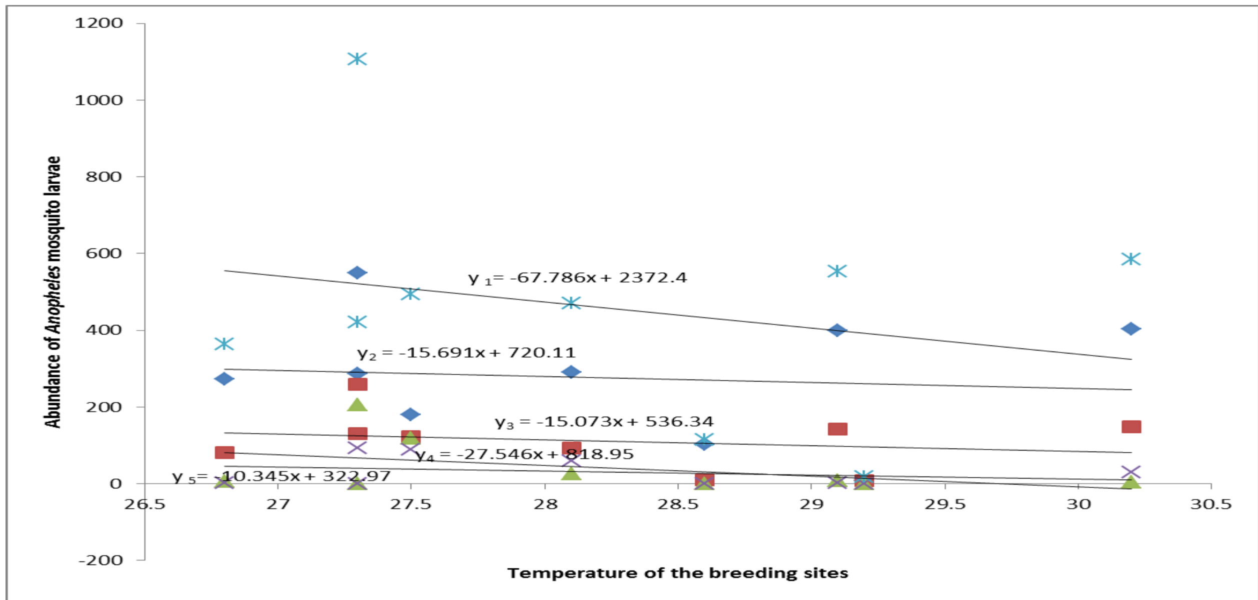


Figure 9: Relationship between *Anopheles* mosquito larval abundance and temperature of the breeding habitats.

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus.*, y_4 for *An. moucheti* and y_5 for *An. nili*.

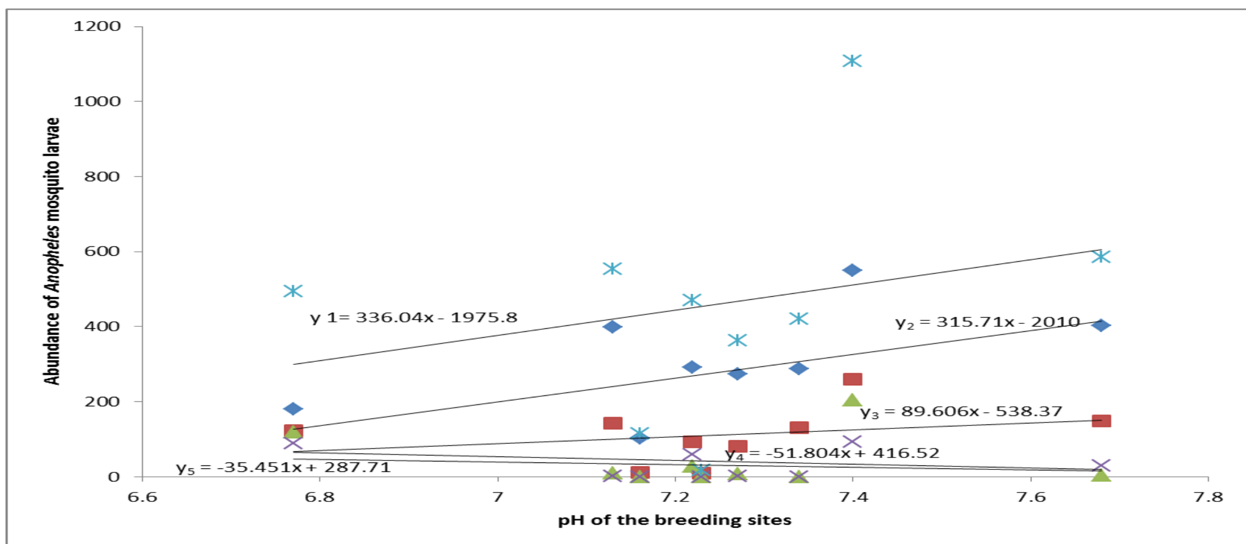


Figure 10: Relationship between *Anopheles* mosquito larval abundance and pH of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus.*, y_4 for *An. moucheti* and y_5 for *An. nili*.

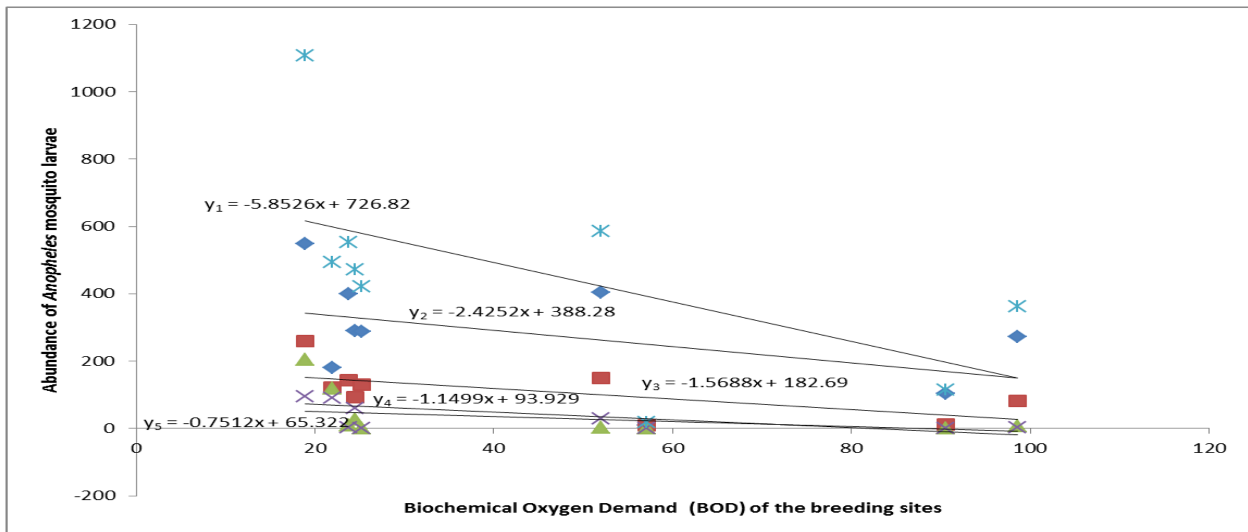


Figure 11: Relationship between Anopheles mosquito larval abundance and Biochemical Oxygen Demand (BOD) of the breeding habitats.

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

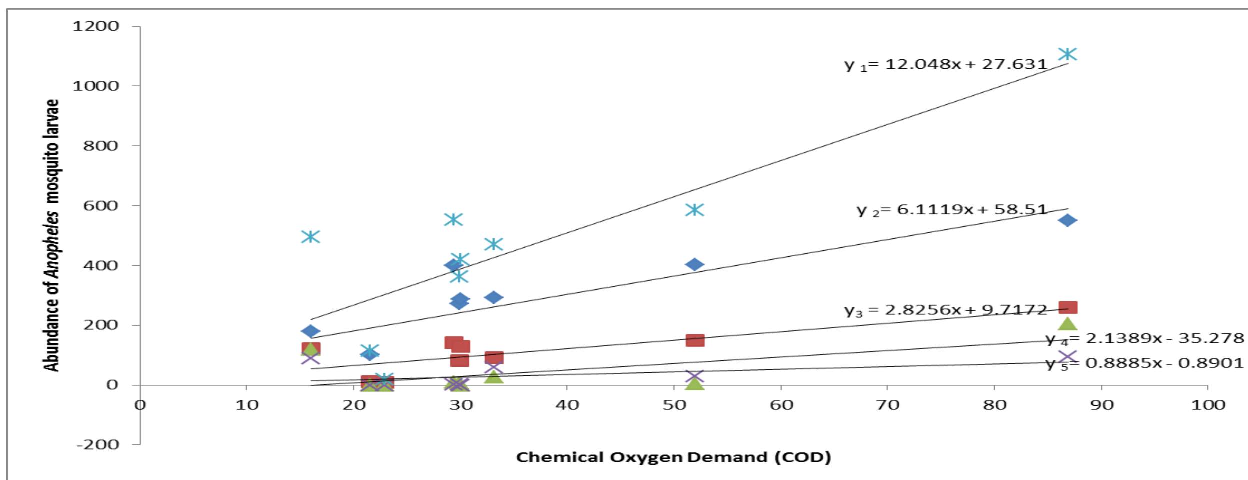


Figure 12: Relationship between *Anopheles* mosquito larval abundance and Chemical Oxygen Demand (COD) of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

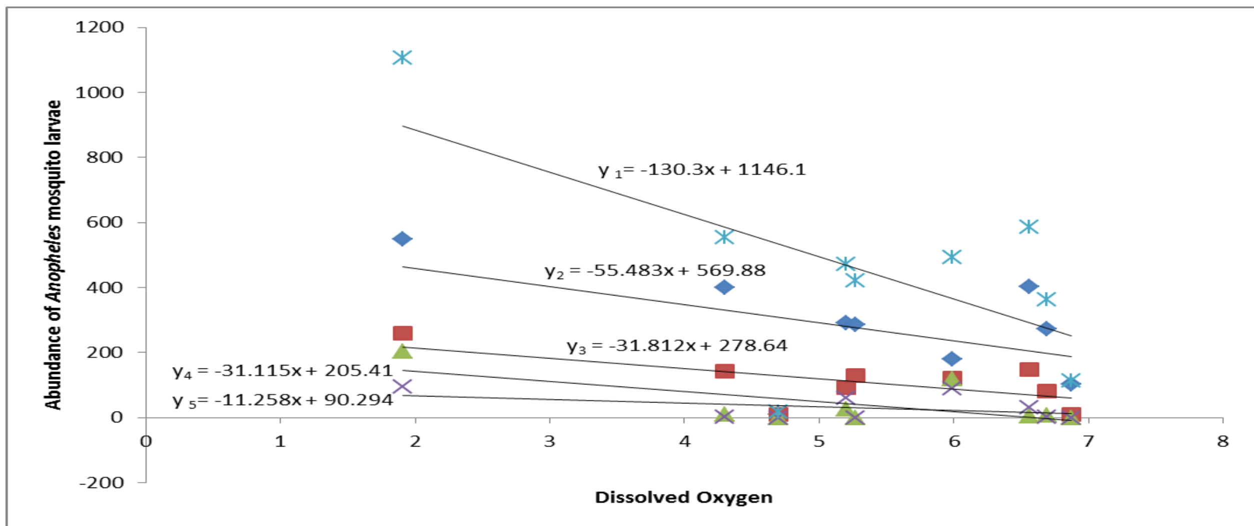


Figure 13: Relationship between Anopheles mosquito larval abundance and Dissolved Oxygen (DO) of the breeding habitats.

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

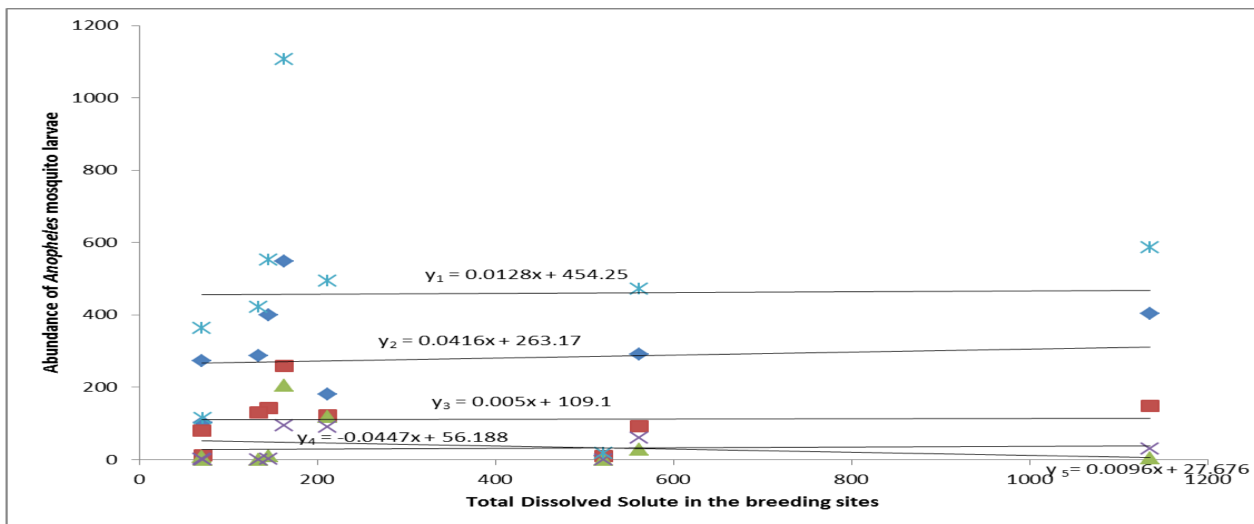


Figure 14: Relationship between *Anopheles* mosquito larval abundance and Total Dissolved Solute (TDS) of the breeding habitats.

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

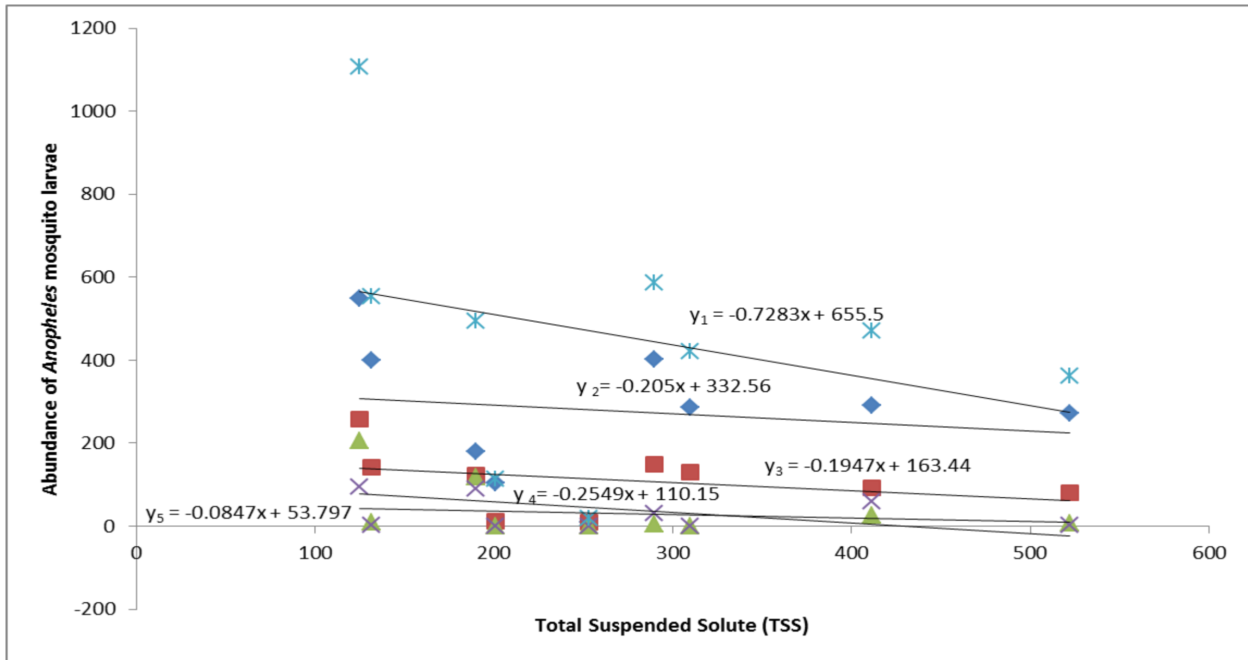


Figure 15: Relationship between *Anopheles* mosquito larval abundance and Total Suspended Solute (TSS) of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

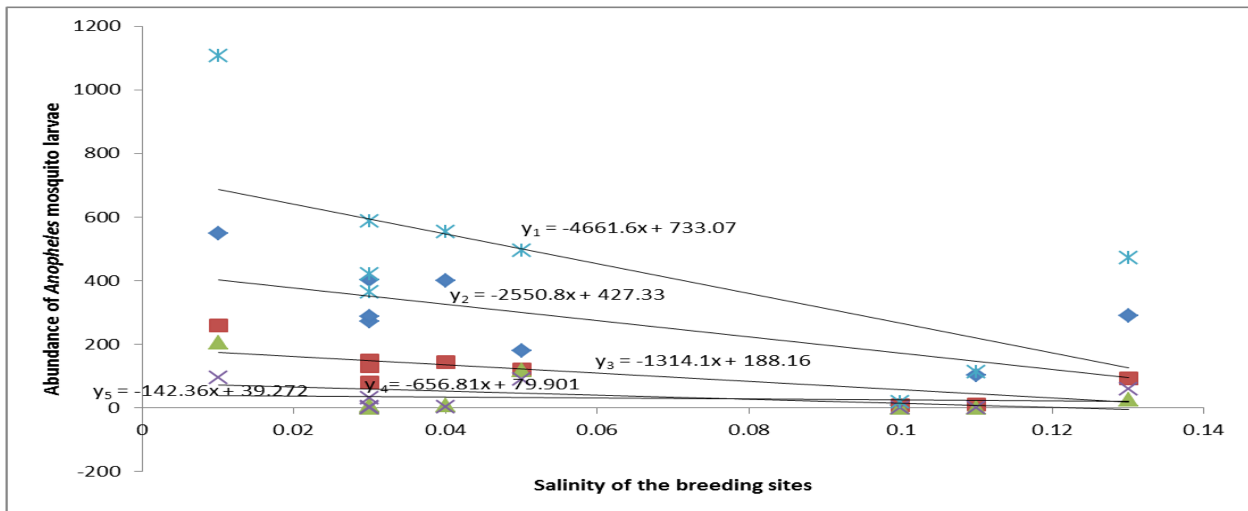


Figure 16: Relationship between *Anopheles* mosquito larval abundance and salinity of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

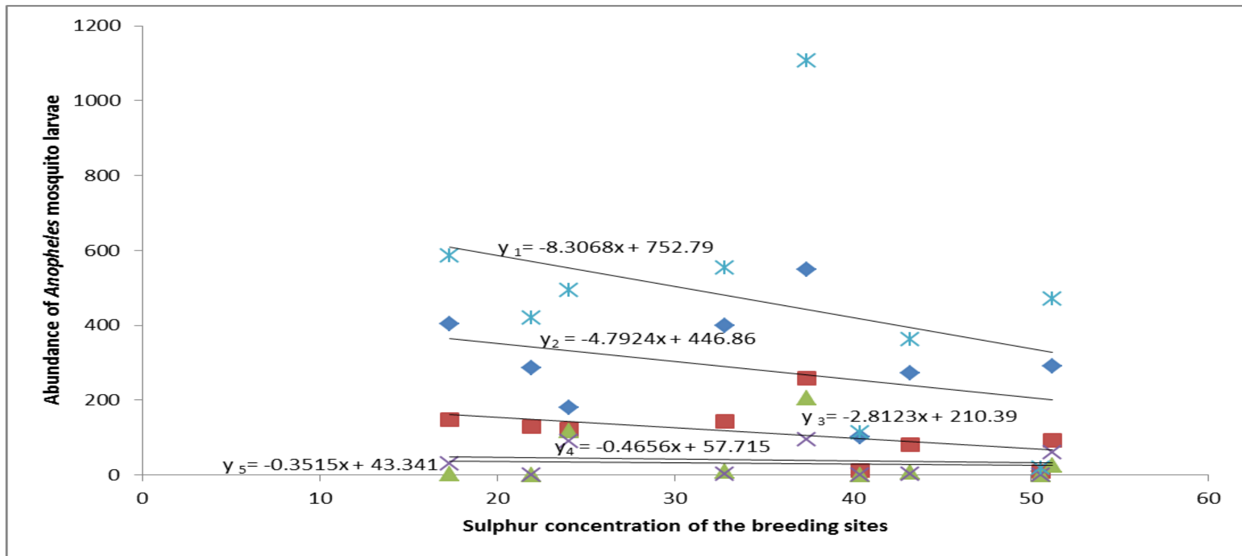


Figure 17: Relationship between *Anopheles* mosquito larval abundance and sulphur concentration of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

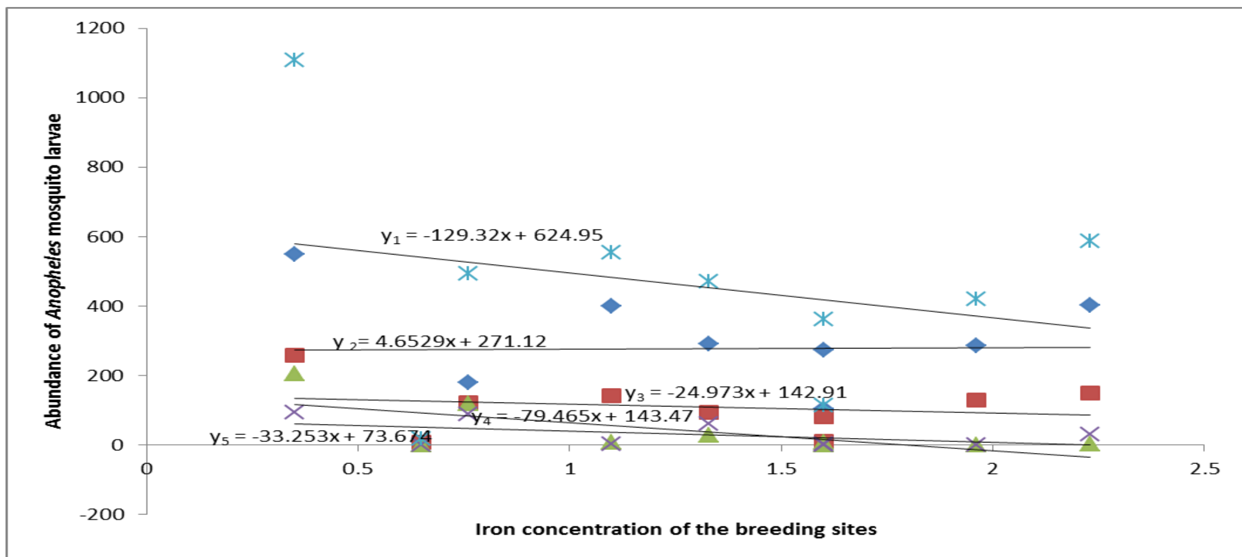


Figure 18: Relationship between *Anopheles* mosquito larval abundance and Iron concentration of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

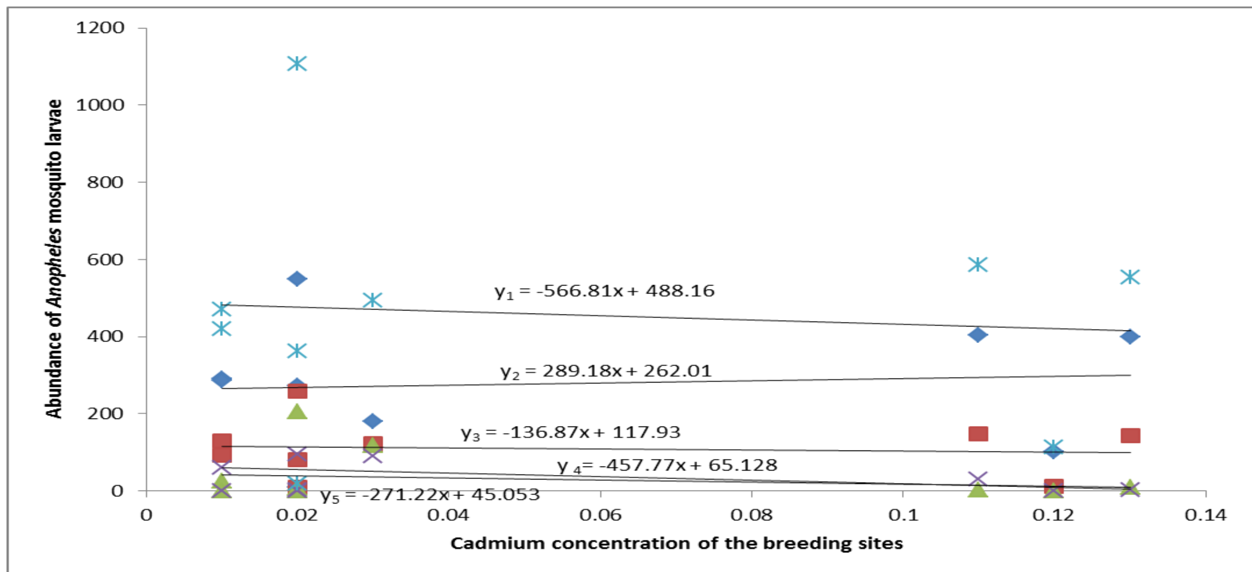


Figure 19: Relationship between *Anopheles* mosquito larval abundance and cadmium concentration of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

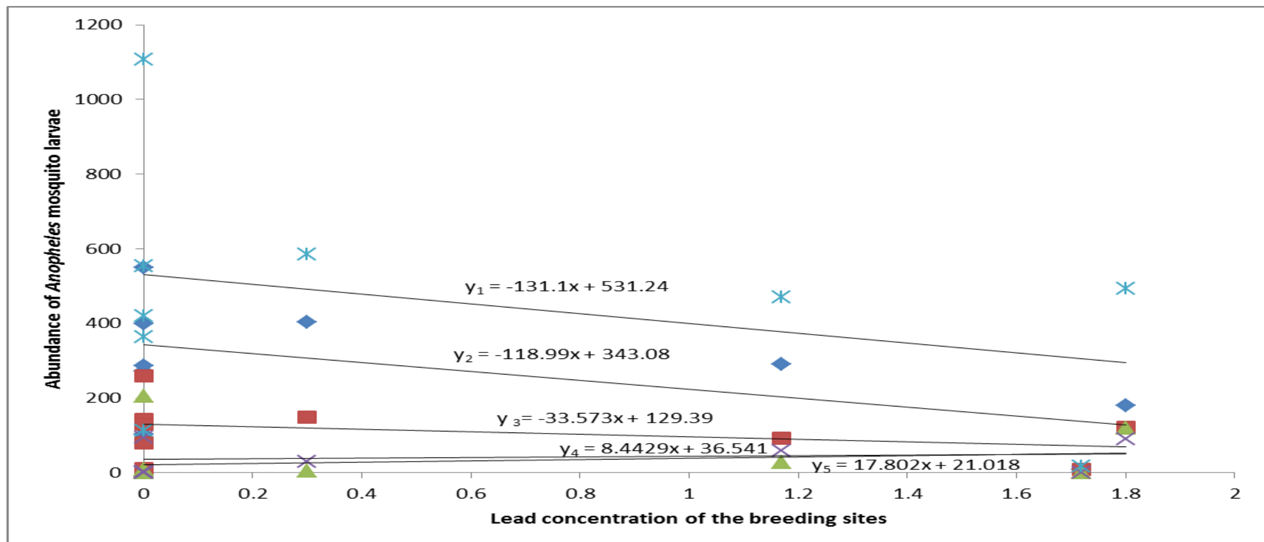


Figure 20: Relationship between *Anopheles* mosquito larval abundance and lead concentration of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

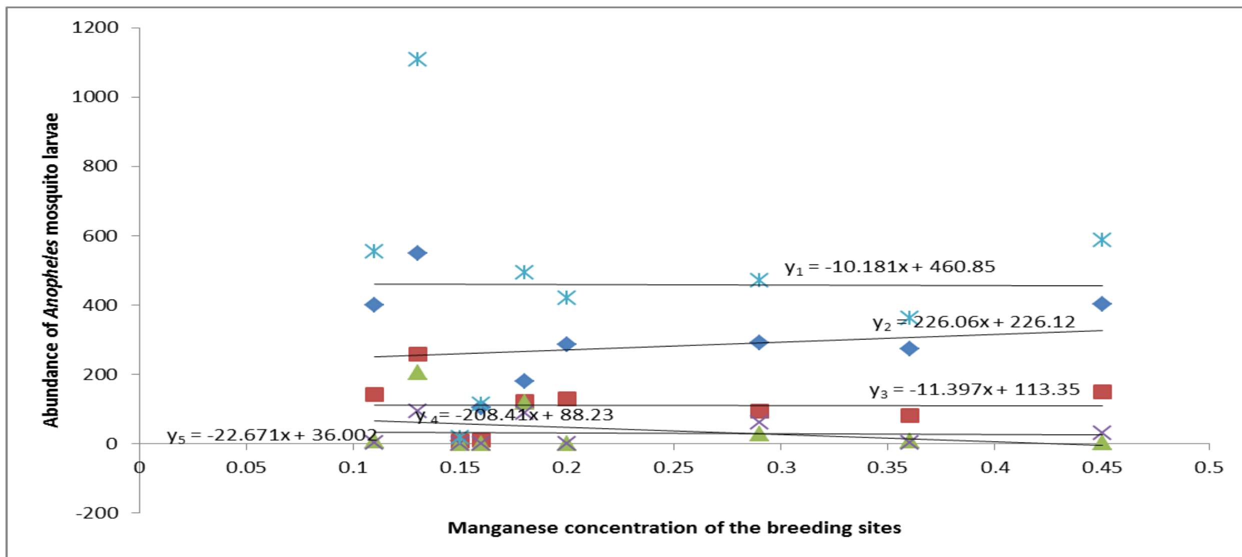


Figure 21: Relationship between *Anopheles* mosquito larval abundance and manganese concentration of the breeding habitats

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

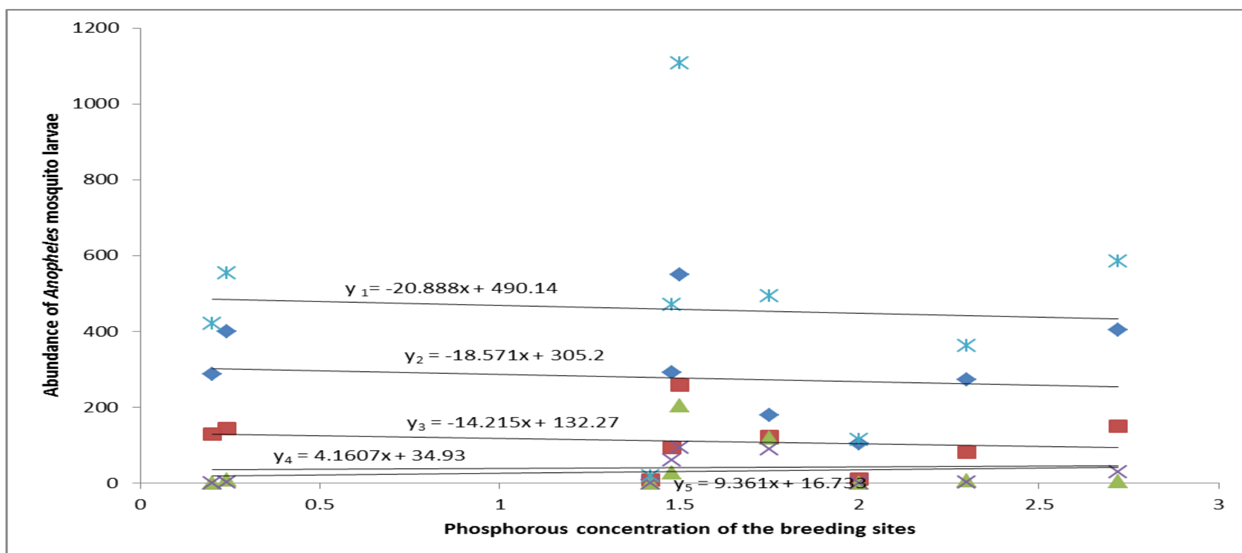


Figure 22: Relationship between *Anopheles* mosquito larval abundance and sulphur concentration of the breeding habitats.

Line y_1 is for total *Anopheles* mosquito, y_2 for *An. gambiae* s. s., y_3 for *An. funestus*., y_4 for *An. moucheti* and y_5 for *An. nili*.

4.3 Biting and resting behavior of the adult *Anopheles* mosquitoes to identify their preferred biting time, biting location and resting location.

4.3.1. Indoor and outdoor abundance of adult *Anopheles* mosquitoes in relation to season

Indoor and outdoor abundance of *Anopheles* mosquitoes are shown in Table 18. A total of 4054 adult female *Anopheles* mosquitoes were collected from the study. Of this number, 3175 (78.3%) were collected indoors and 879 (21.7%) were collected outdoors. There was significant difference between indoor and outdoor collections (P = 0.000, Appendix 35). Four species namely *An. gambiae* s. s., *An. funestus, An. moucheti* and *Anopheles nili* were collected. Indoor collections of these species were 2582 (81.3%) *An. gambiae* s. s., 355 (11.2%) *An. funestus,* 107 (3.4%) *Anopheles moucheti* and 131 (4.1%) *An. nili.* There was significant difference in the abundance of different *Anopheles* species collected indoor in the study area (P = 0.000; Appendix 36). Outdoor collections of these species were 658 (74.9%) *An. gambiae* s. s., 131 (14.9%) *An. funestus,* 41 (4.7%) *An. moucheti* and 49 (5.6%) *An. nili.* There was also significant difference in the outdoor abundance of different *Anopheles* species in the study area (P = 0.00; Appendix 37).

A total of 1415 (34.9%) adult female *Anopheles* mosquitoes were collected during the dry season and 2639 (65.1%) were collected during the wet season. There was no significant difference between dry season and wet season collections (P = 0.061, Appendix 38).

Of the 3175 adult *Anopheles* mosquitoes collected indoor, 1109 (34.9%) were collected during the dry season whereas 2066 (65.1%) were collected during the wet season. In indoor location, during the dry season, the mean±se abundance of the *Anopheles* mosquitoes was 221.8±29.7. The species composition and abundance of *Anopheles* mosquitoes collected indoors during the dry season included: 894 (80.6%) *An. gambiae* s. s., 127 (11.5%) *An. funestus,* 45 (4.1%) *An. moucheti,* and 43 (3.9%) *An. nili*. There was significant difference in the indoor abundance of different *Anopheles* species during the dry season (P = 0.00; Appendix 39).

In indoor location, during the wet season, the mean±se abundance of the *Anopheles* mosquitoes was 295.1±27.3. The species composition and abundance of *Anopheles* mosquitoes collected indoors during the wet season included: 1688 (81.7%) *An. gambiae* s. s., 228 (11.0%) *An. funestus,* 62 (3.0%) *An. moucheti* and 88 (4.3%) *An. nili*. There was significant difference in the indoor abundance of different *Anopheles* species during the wet season (P = 0.00; Appendix 40).

Of the 879 adult *Anopheles* mosquitoes collected outdoor, 306 (34.8%) were collected during the dry season whereas 573 (65.2%) were collected during the wet season. In outdoor location during the dry season, the mean±se abundance of the *Anopheles* mosquitoes was 61.2±7.8. The species composition and abundance of *Anopheles* mosquitoes collected outdoors during the dry season included: 230 (75.2%) *An. gambiae* s. s., 43 (14.1%), *An. funestus,* 15 (4.9%) *An. moucheti,* and 18 (5.9%) *An. nili*. There was significant difference in the outdoor abundance of different *Anopheles* species during the dry season (P = 0.00; Appendix 41).

In outdoor location during the wet season, the mean±se abundance of the *Anopheles* mosquitoes was 81.9±9.2. The species composition and abundance of *Anopheles* mosquitoes collected outdoors during the wet season included: 428 (74.7%) *An. gambiae* s. s., 88 (15.4%) *An. funestus,* 26 (4.5%) *An. moucheti,* and 31 (5.4%) *An. nili*. There was significant difference in the outdoor abundance of different *Anopheles* species during the wet season (P = 0.00; Appendix 42).

Table 18: Indoor and outdoor abundance of adult *Anopheles* mosquitoes in relation to seasons.

Anopheles species	Indoor collection			Outdoor collection			Total		
	Dry season (%)	Wet season (%)	Total	Dry season (%)	Wet season (%)	Total	Dry season (%)	Wet season (%)	Total
Anopheles gambiae s. s.	894 (34.6)	1688 (65.4)	2582	230 (35.0)	428 (65.0)	658	1124 (34.7)	2116 (65.3)	3240
Anopheles funestus	127 (35.8)	228 (64.2)	355	43 (31.4)	94 (68.6)	137	170 (34.6)	322 (65.4)	492
Anopheles moucheti	45 (42.1)	62 (57.9)	107	15 (42.9)	20 (57.1)	35	60 (42.3)	82 (57.7)	142
Anopheles nili	43 (32.8)	88 (67.2)	131	18 (36.7)	31 (63.3)	49	61 (33.9)	119 (66.1)	180
Total	1109 (34.9)	2066 (65.1)	3175	306 (34.8)	573 (65.2)	879	1415 (34.9)	2639 (65.1)	4054

4.3.2 Biting time of *Anopheles* mosquitoes

The hourly biting time of *Anopheles* mosquitoes is shown in Figure 23. The peak biting time for *Anopheles* mosquitoes was 2.00am - 3.00am with 14.6% (128/879) of *Anopheles* mosquitoes collected. The peak biting time for *An. gambiae* s. s. was 2.00am - 3.00am with 15.0% (99/658) of *An. gambiae* s. s. collected. The peak biting times for *An. funestus* were 10.00pm – 11.00pm and 2.00am - 3.00am with 16.8% (23/137) of *An. funestus* collected. The peak biting time for *An. moucheti* was 11.00pm - 12.00am with 20.0% (7/35) of *An. moucheti* collected. The peak biting time for *An. nili* was 12.00am - 1.00am with 18.4% (9/49) of *An. nili* collected.

The monthly biting time of *Anopheles* mosquitoes is shown in Figure 24. The peak monthly biting time for *Anopheles* mosquitoes was June with 12.6% (111/879) of *Anopheles* mosquitoes collected. The peak biting time for *An. gambiae* s. s. was June with 12.8 % (84/658) of *An. gambiae* s. s. collected. The peak monthly biting times for *An. funestus* was June with 13.1% (18/137) of *An. funestus* collected. The peak monthly biting time for *An. moucheti* was April with 17.1% (6/35) of *An. moucheti* collected. The peak monthly biting time for *An. nili* was July with 16.3% (8/49) of *An. nili* collected.

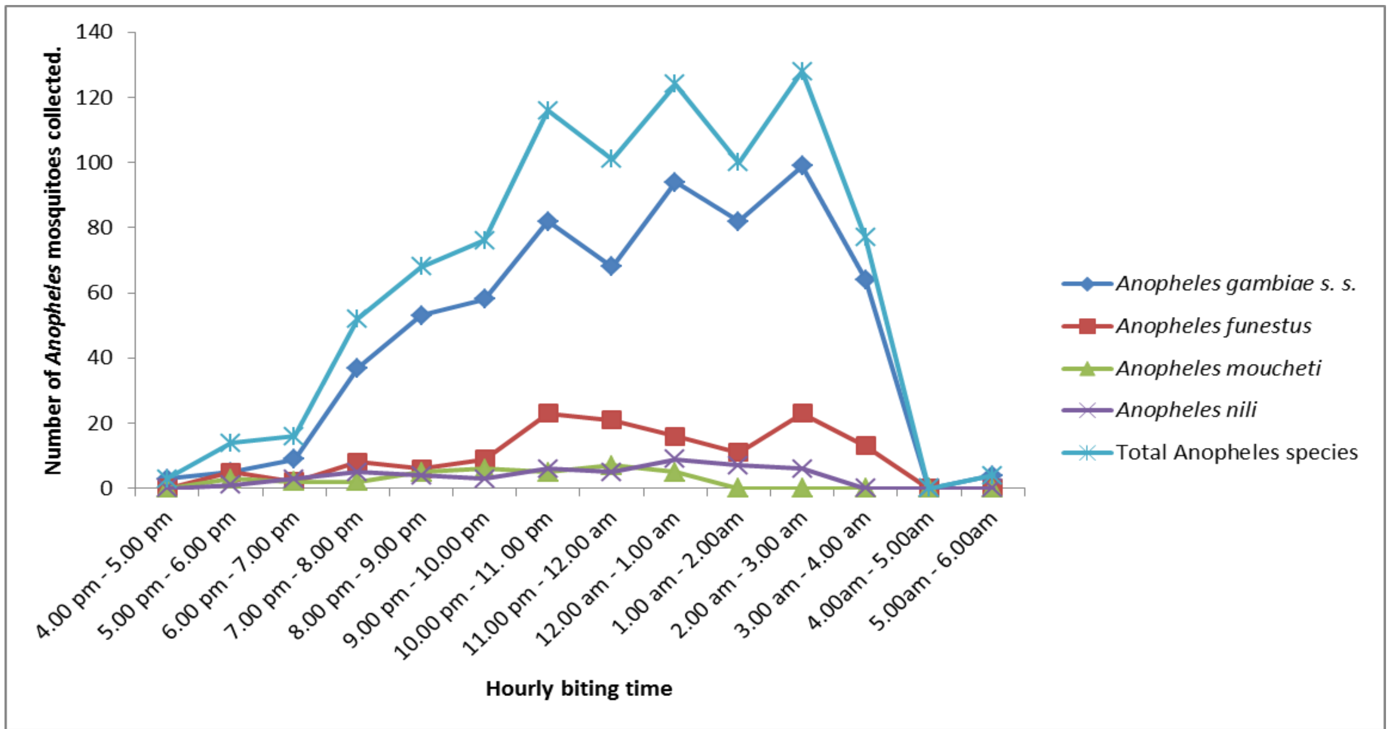


Figure 23 : Hourly biting time of different *Anopheles* species

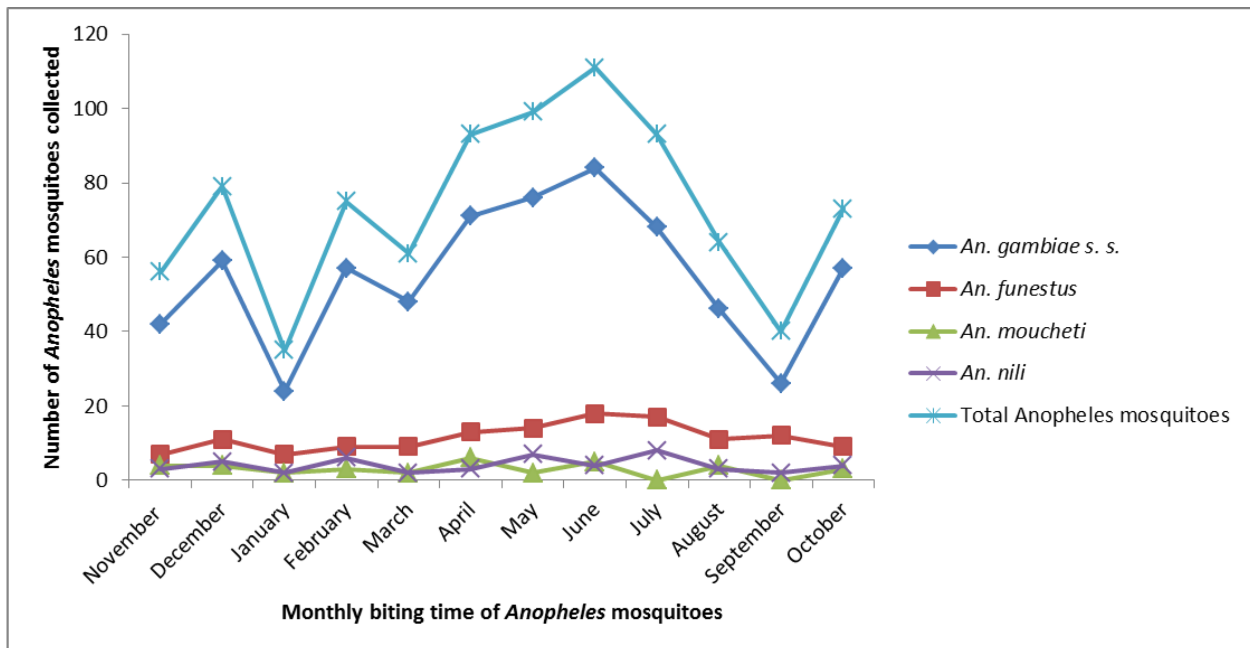


Figure 24 : Monthly biting time of different *Anopheles* species

4.3.3 Biting and resting locations of *Anopheles* mosquitoes

The biting and resting locations of *Anopheles* mosquitoes were determined using their gonotrophic states. Out of 3175 adult *Anopheles* mosquitoes collected indoors, 1986 (62.6%) were fed, 258 (8.1%) were gravid, 519 (16.3%) were half gravid and 412 (13.0%) were unfed (Table 19). There was significant difference in the number of *Anopheles* mosquitoes in the different gonotrophic states (P = 0.000; Appendix 43). *An. funestus* had the highest proportion of fed mosquitoes with 65.1% (231/355) record. *An. nili* had the highest proportion of gravid mosquitoes with 12.2% (16/131) record. *An. moucheti* had the highest proportion of half gravid mosquitoes with 22.4% (24/107) record. *An. nili* had the highest proportion of unfed mosquitoes with 15.3% (20/131) record.

Table 19: Gonotrophic state of *Anopheles* species collected indoors from the study.

Anopheles species	Fed (%)	Gravid (%)	Half gravid (%)	Unfed (%)	Total
Anopheles gambiae s. s.	1616 (62.6)	195 (7.6)	428 (16.6)	343 (13.3)	2582
Anopheles funestus	231 (65.1)	39 (11.0)	50 (14.1)	35 (9.9)	355
Anopheles moucheti	61 (57.0)	8 (7.5)	24 (22.4)	14 (13.1)	107
Anopheles nili	78 (59.5)	16 (12.2)	17 (13.0)	20 (15.3)	131
Total	**1986 (62.6)**	**258 (8.1)**	**519 (16.3)**	**412 (13.0)**	**3175**

4.3.3.1 Biting location of *Anopheles* species

Endophagic (P_1) and exophagic (P_O) indices of *Anopheles* species in outdoor and indoor locations were used to determine their preferred biting location (Table 20). The overall endophagic index of *Anopheles* mosquitoes was 66.2% whereas the overall exophagic index was 33.8%. The difference in these biting indices of *Anopheles* mosquitoes was statistically significant (P = 0.000; Appendix 44). During the dry season, *Anopheles* mosquitoes recorded significantly higher P_1, 68.8% than P_O, 31.2% (P = 0.024; Appendix 45). During the wet season, *Anopheles* mosquitoes recorded significantly higher P_1, 65.8% than P_O, 34.2% (P = 0.001; Appendix 46).

The endophagic index of *An. gambiae* s. s was 69.0% whereas the exophagic index was 31.0%. The difference in these biting indices of *An. gambiae* s. s was statistically significant (P = 0.000; Appendix 47). During the dry season, *An. gambiae* s. s. recorded significantly higher P_1, 70.0% than P_O, 30.0% (P = 0.025; Appendix 48). During the wet season, *An. gambiae* s. s. recorded significantly higher P_1, 68.3% than P_O, 31.7% (P = 0.001; Appendix 49).

The endophagic index of *An. funestus* was 60.0% whereas the exophagic index was 40.0%. The difference in these biting indices of *An. funestus* was statistically significant (P = 0.001; Appendix 50). During the dry season, *An. funestus* recorded significantly higher P_1, 62.5% than P_O, 37.5% (P = 0.008; Appendix 51). During the wet season, *An. funestus* recorded significantly higher P_1, 60.0% than P_O, 40.0% (P = 0.047; Appendix 52).

The endophagic index of *An. moucheti* was 50.0% and the exophagic index was also 50.0%. The was no significant difference in these biting indices of *An. moucheti* (P = 0.052; Appendix 53). During the dry season, *An. moucheti* recorded higher P_1, 66.7% than P_O, 33.3%. However, the difference was not statistically significant (P = 0.132; Appendix 54). During the wet season, the P_1, and P_O records of *An. moucheti* were equal, 50.0% (P = 0.195; Appendix 55).

The endophagic index of *An. nili* was 66.7% whereas the exophagic index was 33.3%. The difference in these biting indices of *An. nili* was not statistically significant (P = 0.200, Appendix 56). During the dry season, *An. nili* recorded higher P_1, 66.7% than P_O, 33.3%. However, the difference was not statistically significant (P = 0.623; Appendix 57). During the wet season, *An.*

nili recorded higher P_1, 66.7% than P_O, 33.3%. However, the difference was not statistically significant (P = 0.202; Appendix 58).

Table 20: Human biting rates, endophagic and exophagic indices of *Anopheles* species with respect to seasons.

Anopheles species	**Dry season**				**Wet season**				**Total**			
	Indoor		Outdoor		Indoor		Outdoor		Indoor		Outdoor	
	MBR_I	P_I (%)	MBR_O	P_O (%)	MBR_I	P_I (%)	MBR_O	P_O (%)	MBR_I	P_I (%)	MBR_O	P_O (%)
An. gambiae s. s.	3.5	70.0	1.5	30.0	4.3	68.3	2.0	31.7	4.0	69.0	1.8	31.0
An. funestus	0.5	62.5	0.3	37.5	0.6	60.0	0.4	40.0	0.6	60.0	0.4	40.0
An. moucheti	0.2	66.7	0.1	33.3	0.1	50.0	0.1	50.0	0.1	50.0	0.1	50.0
An. nili	0.2	66.7	0.1	33.3	0.2	66.7	0.1	33.3	0.2	66.7	0.1	33.3
Total	4.4	68.8	2.0	31.2	5.2	65.8	2.7	34.2	4.9	66.2	2.4	33.8

MBR_I = Human biting rate in indoor location
P_I = Endophagic index
MBR_O = Human biting rate in outdoor location
P_O = Exophagic index

4.3.3.2 Post feeding resting location of *Anopheles* mosquitoes

The proportions of gravid *Anopheles* mosquitoes in relation to the number of blood fed *Anopheles* mosquitoes indoors were used to determine their preferred resting location (Table 21). The overall indoor proportion of gravid *Anopheles* mosquitoes was 39.2% while the overall outdoor proportion (number of *Anopheles* mosquitoes exiting houses after feeding) was 60.8%. However, there was no significant difference in the indoor and outdoor proportions of gravid *Anopheles* mosquitoes (P = 0.050; Appendix 59). During the dry season, significantly higher proportion, 68.4% of gravid *Anopheles* mosquitoes was found outdoor than in indoor location with 31.6% (P = 0.029; Appendix 60). During the wet season, higher proportion, 56.4% of gravid *Anopheles* mosquitoes was found outdoor than in indoor location with 43.6%; and the difference was not statistically significant (P = 0.397; Appendix 61).

The overall indoor proportion of gravid *An. gambiae* s. s. was 38.5% while the overall outdoor proportion was 61.5%. However, there was no significant difference in the indoor and outdoor proportions of gravid *An. gambiae* s. s. (P = 0.052; Appendix 62). During the dry season, significantly higher proportion, 68.8% of gravid *An. gambiae* s. s. was found outdoor than in indoor location with 31.2% (P = 0.029; Appendix 63). During the wet season, higher proportion, 57.3% of gravid *An. gambiae* s. s. was found outdoor than in indoor location with 42.7% and the difference was not statistically significant (P = 0.393; Appendix 64).

The overall indoor proportion of gravid *An. funestus* was 38.3% while the overall outdoor proportion was 61.7%; and the difference was statistically significant (P = 0.018; Appendix 65). During the dry season, significantly higher proportion, 68.6% of gravid *An. funestus* was found outdoor than in indoor location with 31.4% (P = 0.023; Appendix 66). During the wet season, higher proportion, 57.0% of gravid *An. funestus* was found outdoor than in indoor location with 43.0%; and the difference was not statistically significant (P = 0.286; Appendix 67).

The overall indoor proportion of gravid *An. moucheti* was 54.0% while the overall outdoor proportion was 46.0%. However, there was no significant difference in the indoor and outdoor proportions of gravid *An. moucheti* (P = 0.485; Appendix 68). During the dry season, higher

proportion, 58.6% of gravid *An. moucheti* was found outdoor than in indoor location with 41.4%; and the difference was not statistically significant (P = 0.780; Appendix 69). During the wet season, higher proportion, 63.0% of gravid *An. moucheti* was found indoor than in outdoor location with 37.0%; and the difference was equally not statistically significant (P = 0.298; Appendix 70).

The overall indoor proportion of gravid *An. nili* was 43.1% while the overall outdoor proportion was 56.9%. However, there was no significant difference in the indoor and outdoor proportions of gravid *An. nili* (P = 0.806; Appendix 71). During the dry season, higher proportion, 71.4% of gravid *An. nili* was found outdoor than in indoor location with 28.6%; and the difference was not statistically significant (P = 0.152; Appendix 72). During the wet season, higher proportion, 50.7% of gravid *An. nili* was found indoor than in outdoor location with 49.3%; and the difference was equally not statistically significant (P = 0.507; Appendix 73).

Table 21: Mean Fed, mean gravid, indoor resting proportion and outdoor resting proportion of *Anopheles* species with respect to seasons.

Anopheles species	Dry season				Wet season				Total			
	Blood fed	Total gravid	P_I (%)	P_O (%)	Blood fed	Total gravid	P_I (%)	P_O (%)	Blood fed	Total gravid	P_I (%)	P_O (%)
An. gambiae s. s.	117.8±17.3	36.8±7.8	31.2	68.8	146.7±12.0	62.7±15.8	42.7	57.3	134.7±10.5	51.9±10.2	38.5	61.5
An. funestus	17.2±1.2	5.4±0.7	31.4	68.6	20.7±1.6	8.9±1.4	43.0	57.0	19.3±1.1	7.4±1.0	38.3	61.7
An. moucheti	5.8±1.0	2.4±1.0	41.4	58.6	4.6±1.0	2.9±0.7	63.0	37.0	5.0±0.7	2.7±0.5	54.0	46.0
An. nili	5.6±1.8	1.6±0.4	28.6	71.4	7.1±1.5	3.6±0.6	50.7	49.3	6.5±1.1	2.8±0.5	43.1	56.9
Total	146.4±19.6	46.2±8.0	31.6	68.4	179.1±12.6	78.0±17.9	43.6	56.4	165.5±11.5	64.8±11.6	39.2	60.8

P_I = Indoor proportion of gravid *Anopheles* mosquitoes
P_O = 1 - P_I = Outdoor proportions of gravid *Anopheles* mosquitoes (proportion of *Anopheles* mosquitoes exiting house after feeding).

4.4 Entomological indices (Human Blood Index, Sporozoite Rate and Entomological Inoculation Rate) of *Anopheles* mosquitoes to ascertain malaria transmission in the study area.

4.4.1 Human Blood Index of *Anopheles* mosquitoes

Table 22 shows the human blood indices of *Anopheles* mosquitoes collected from the study. Out of the 1986 fed *Anopheles* mosquitoes collected, 1886 (95.0%) had human blood. Proportion of *Anopheles* mosquitoes that had human blood was higher in the wet season (95.4%) than in the dry season (94.3%). There was no significant difference in the proportion of *Anopheles* mosquitoes that had human blood between the wet and the dry season (P = 0.418; Appendix 74). The month of October recorded the highest proportion, 99.1% of *Anopheles* mosquitoes that had human blood whereas the month of June recorded the least proportion, 91.3%. There was no significant difference in the proportions of *Anopheles* mosquitoes that had human blood in the different months (P = 0.968; Appendix 75).

Of all *Anopheles* species collected, *An. gambiae* s. s. recorded the highest proportion (97.4%) that had human blood whereas *An. moucheti* recorded the least proportion (75.4%). There was significant difference in the proportion of different *Anopheles* species that had human blood (P = 0.002; Appendix 76). In the dry season, *An. gambiae* s. s. recorded the highest proportion (97.3%) that had human blood whereas *An. nili* recorded the least proportion (78.6%). There was no significant difference in the proportion of different *Anopheles* species that had human blood in the dry season (P = 0.054; Appendix 77). In the wet season, *An. gambiae* s. s. recorded the highest proportion (97.5%) that had human blood whereas *An. moucheti* recorded the least proportion (71.9%). There was significant difference in the proportion of different *Anopheles* species that had human blood in the wet season (P = 0.049; Appendix 78).

Out of 1616 fed *An. gambiae* s. s., 1574 (97.4%) had human blood. In the dry season, 97.3% (573/589) *An. gambiae* s. s. had human blood. In the wet season, 97.5% (1001/1027) *An. gambiae* s. s. had human blood. There was no significant difference in the proportion of *Anopheles gambiae* s. s. that had human blood in the wet and dry seasons (P = 0.988; Appendix 79).

Out of 231 fed *An. funestus*, 201 (87.0%) had human blood. In the dry season, 82.7% (72/86) *Anopheles funestus* had human blood. In the wet season, 89.0% (129/145) *An. funestus* had human blood. There was no significant difference in the proportion of *An. funestus* that had human blood in the wet and dry seasons (P = 0.266; Appendix 80).

Out of 61 fed *An. moucheti*, 46 (75.4%) had human blood. In the dry season, 79.3% (23/29) *An. moucheti* had human blood. In the wet season, 71.9% (23/32) *An. moucheti* had human blood. There was no significant difference in the proportion of *An. moucheti* that had human blood in the wet and dry seasons (P = 0.897; Appendix 81).

Out of 78 fed *An. nili*, 65 (83.3%) had human blood. In the dry season, 78.6% (22/28) *An. nili* had human blood. In the wet season, 86.0% (43/50) *An. nili* had human blood. There was no significant difference in the proportion of *An. nili* that had human blood in the wet and dry seasons (P = 0.291; Appendix 82).

Table 22: Human Blood Index of *Anopheles* species collected from the study

Month	*Anopheles gambiae* s. s		*Anopheles funestus*		*Anopheles moucheti*		*Anopheles nili*		Total	
	No. fed	**HBI (%)**	**No. fed**	**HBI (%)**	**No. fed**	**HBI (%)**	**No. fed**	**HBI (%)**	**No. fed**	**HBI (%)**
November	67	67 (100.0)	14	11 (78.6)	3	2 (66.7)	3	3 (100.0)	87	83 (95.4)
December	87	83 (95.4)	18	14 (77.8)	8	7 (87.5)	3	2 (66.7)	116	106 (91.4)
January	157	149 (94.9)	20	19 (95.0)	7	7 (100.0)	12	10 (83.3)	196	185 (94.4)
February	141	140 (99.3)	15	13 (86.7)	4	2 (50.0)	7	5 (71.4)	167	160 (95.8)
March	137	134 (97.8)	19	15 (78.9)	7	5 (71.4)	3	2 (66.7)	166	156 (94.0)
Total (dry)	**589**	**573 (97.3)**	**86**	**72 (83.7)**	**29**	**23 (79.3)**	**28**	**22 (78.6)**	**732**	**690 (94.3)**
April	120	120 (100.0)	19	15 (78.9)	4	3 (75.0)	12	11 (91.7)	155	149 (96.1)
May	190	185 (97.4)	27	23 (85.2)	5	5 (100.0)	7	5 (71.4)	229	218 (95.2)
June	149	138 (92.6)	22	18 (81.8)	8	8 (100.0)	5	4 (80.0)	184	168 (91.3)
July	121	116 (95.9)	19	16 (84.2)	1	1 (100.0)	12	10 (83.3)	153	143 (93.5)
August	121	119 (98.3)	15	14 (93.3)	4	2 (50.0)	4	3 (75.0)	144	138 (95.8)
September	135	133 (98.5)	25	25 (100.0)	8	3 (37.5)	2	2 (100.0)	170	163 (95.9)
October	191	190 (99.5)	18	18 (100.0)	2	1 (50.0)	8	8 (100.0)	219	217 (99.1)
Total (wet)	1027	1001 (97.5)	145	129 (89.0)	32	23 (71.9)	50	43 (86.0)	1254	1196 (95.4)
Grand Total	**1616**	**1574 (97.4)**	**231**	**201 (87.0)**	**61**	**46 (75.4)**	**78**	**65 (83.3)**	**1986**	**1886 (95.0)**

4.4.2. Sporozoite rate of *Anopheles* mosquitoes

Table 23 shows the sporozoite rates of *Anopheles* mosquitoes collected from the study. Out of the 1886 *Anopheles* mosquitoes that fed on human blood, 48 (2.5%) were positive with *P. falciparum* sporozoite. Proportion of *Anopheles* mosquitoes that were positive with *P. falciparum* sporozoite was higher in the dry season (3.3%) than in the wet season (2.1%). There was no significant difference in the proportion of *Anopheles* mosquitoes that were positive with *P. falciparum* sporozoite in the wet and dry seasons (P = 0.338; Appendix 83). The month of July recorded the highest proportion, 5.6% of *Anopheles* mosquitoes that were positive with *P. falciparum* sporozoite whereas the month of June recorded the least proportion, 0.6%. There was no significant difference in the proportion of *Anopheles* mosquitoes that were positive with *P. falciparum* sporozoite in the different months (P = 0.181; Appendix 84).

Of all *Anopheles species* collected, *An. funestus* recorded the highest proportions (3.5%) that were positive with *P. falciparum* sporozoite whereas *An. nili* recorded the least proportion (1.5%). There was no significant difference in the proportion of different *Anopheles* species that were positive with *P. falciparum* sporozoite (P = 0.153; Appendix 85). In the dry season, *An. nili* recorded the highest proportions (4.5%) that were positive with *P. falciparum* sporozoite whereas *An. funestus* recorded the least proportion (2.8%). There was no significant difference in the proportion of different *Anopheles* species that were positive with *P. falciparum* sporozoite in the dry season (P = 0.926; Appendix 86). In the wet season, *An. funestus* recorded the highest proportion (3.9%) that was positive with *P. falciparum* sporozoite whereas *An. moucheti* and *An. nili* recorded the least proportion (0.0%). There was significant difference in the proportion of different *Anopheles* species that were positive with *P. falciparum* sporozoite in the wet season (P = 0.001; Appendix 87).

Out of 1574 *An. gambiae* s. s. that fed on human blood, 39 (2.5%) were positive with *P. falciparum* sporozoite. In the dry season, 3.3% (19/573) *An. gambiae* s. s. was positive with *P. falciparum* sporozoite. In the wet season, 2.0% (20/1001) *An. gambiae* s. s. was positive with *P. falciparum* sporozoite. There was no significant difference in the proportion of *An. gambiae* s. s. were positive with *P. falciparum* sporozoite blood in the wet and dry seasons (P = 0.243; Appendix 88).

Out of 201 *An. funestus* that fed on human blood, (3.5%) were positive with *P. falciparum* sporozoite. In the dry season, 2.8% (2/72) *An. funestus* were positive with *P. falciparum* sporozoite. In the wet season, 3.9% (5/129) *An. funestus* were positive with *P. falciparum* sporozoite. There was no significant difference in the proportion of *An. funestus* that were positive with *P. falciparum* sporozoite in the wet and dry seasons (P = 0.549; Appendix 89).

Out of 46 *An. moucheti* that fed on human blood, 1 (2.2%) was positive with *P. falciparum* sporozoite. In the dry season, 4.3% (1/23) *An. moucheti* was positive with *P. falciparum* sporozoite. In the wet season, 0.0% (0/23) *An. moucheti* was positive with *P. falciparum* sporozoite. There was no significant difference in the proportion of *An. moucheti* that were positive with *P. falciparum* sporozoite in the wet and dry seasons (P = 0.255; Appendix 90).

Out of 65 *An. nili* that fed on human blood, 1 (1.5%) was positive with *P. falciparum* sporozoite. In the dry season, 4.5% (1/22) *An. nili* was positive with *P. falciparum* sporozoite. In the wet season, 0.0% (0/43) *An. nili* was positive with *P. falciparum* sporozoite. There was no significant difference in the proportion of *An. nili* that were positive with *P. falciparum* sporozoite in the wet and dry seasons (P = 0.255; Appendix 91).

Table 23: Sporozoite rate of *Anopheles* species collected from the study.

Month	*Anopheles gambiae* s. s		*Anopheles funestus*		*Anopheles moucheti*		*Anopheles nili*		Total	
	HBI	No. infected (%)	HBI	No. infected (%)	HBI	No. infected (%)	HBI	No. infected (%)	HBI (%)	No. infected (%)
November	67	1 (1.5)	11	0 (0.0)	2	0 (0.0)	3	0 (0.0)	83	1 (1.2)
December	83	4 (8.4)	14	1(7.1)	7	0 (0.0)	2	0 (0.0)	106	5 (4.7)
January	149	7 (4.7)	19	0 (0.0)	7	1 (14.3)	10	1 (10.0)	185	9 (4.9)
February	140	2 (1.4)	13	0 (0.0)	2	0 (0.0)	5	0 (0.0)	160	2 (1.3)
March	134	5 (3.7)	15	1 (6.7)	5	0 (0.0)	2	0 (0.0)	156	6 (3.8)
Total (dry)	573	19 (3.3)	72	2 (2.8)	23	1 (4.3)	22	1 (4.5)	690	23 (3.3)
April	120	2 (1.7)	15	1 (6.7)	3	0 (0.0)	11	0 (0.0)	149	3 (2.0)
May	185	5 (2.7)	23	1 (4.3)	5	0 (0.0)	5	0 (0.0)	218	6 (2.8)
June	138	1 (0.7)	18	0 (0.0)	8	0 (0.0)	4	0 (0.0)	168	1 (0.6)
July	116	8 (6.9)	16	0 (0.0)	1	0 (0.0)	10	0 (0.0)	143	8 (5.6)
August	119	1 (0.8)	14	1 (7.1)	2	0 (0.0)	3	0 (0.0)	138	2 (1.4)
September	133	2 (1.5)	25	1 (4.0)	3	0 (0.0)	2	0 (0.0)	163	3 (1.8)
October	190	1 (0.5)	18	1 (5.6)	1	0 (0.0)	8	0 (0.0)	217	2 (0.9)
Total (wet)	1001	20 (2.0)	129	5 (3.9)	23	0 (0.0)	43	0 (0.0)	1196	25 (2.1)
Grand Total	**1574**	**39 (2.5)**	**201**	**7 (3.5)**	**46**	**1 (2.2)**	**65**	**1 (1.5)**	**1886**	**48 (2.5)**

4.4.2.1 Effect of temperature on the sporozoite rate of *Anopheles* mosquitoes.

The effect of temperature on the sporozoite rates of *Anopheles* mosquitoes is shown in Figure 25 below. There was no significant correlation between the monthly mean temperature and sporozoite rates of *Anopheles* mosquitoes. However, the relationship between temperature and sporozoite rates of *Anopheles* mosquitoes is given by the regression equation: Sporozoite rate = -0.08 temperature + 4.8012 ($R^2 = 0.037$). The relationship between temperature and sporozoite rates of *Anopheles gambiae* s. s. is given by the regression equation: Sporozoite rate = -0.099 temperature + 5.6175 ($R^2 = 0.0026$). The relationship between temperature and sporozoite rates of *Anopheles funestus* is given by the regression equation: Sporozoite rate = 0.1035 temperature + 3.5893 ($R^2 = 0.0018$). The relationship between temperature and sporozoite rates of *Anopheles moucheti* is given by the regression equation: Sporozoite rate = -0.2324 temperature + 7.6322 ($R^2 = 0.0055$). The relationship between temperature and sporozoite rates of *Anopheles nili* is given by the regression equation: Sporozoite rate = -0.1625 temperature + 5.3372 ($R^2 = 0.0055$). However, there was no significant correlation between temperature and sporozoite rate of *Anopheles* mosquitoes (r = -0.061, P = 0.852), *Anopheles gambiae* s. s. (r = -0.051, P = 0.876), *Anopheles funestus* (r = 0.042, P = 0.896) *Anopheles moucheti* (r = -0.074, P = 0.819) and *Anopheles nili* (r = -0.074, P = 0.819).

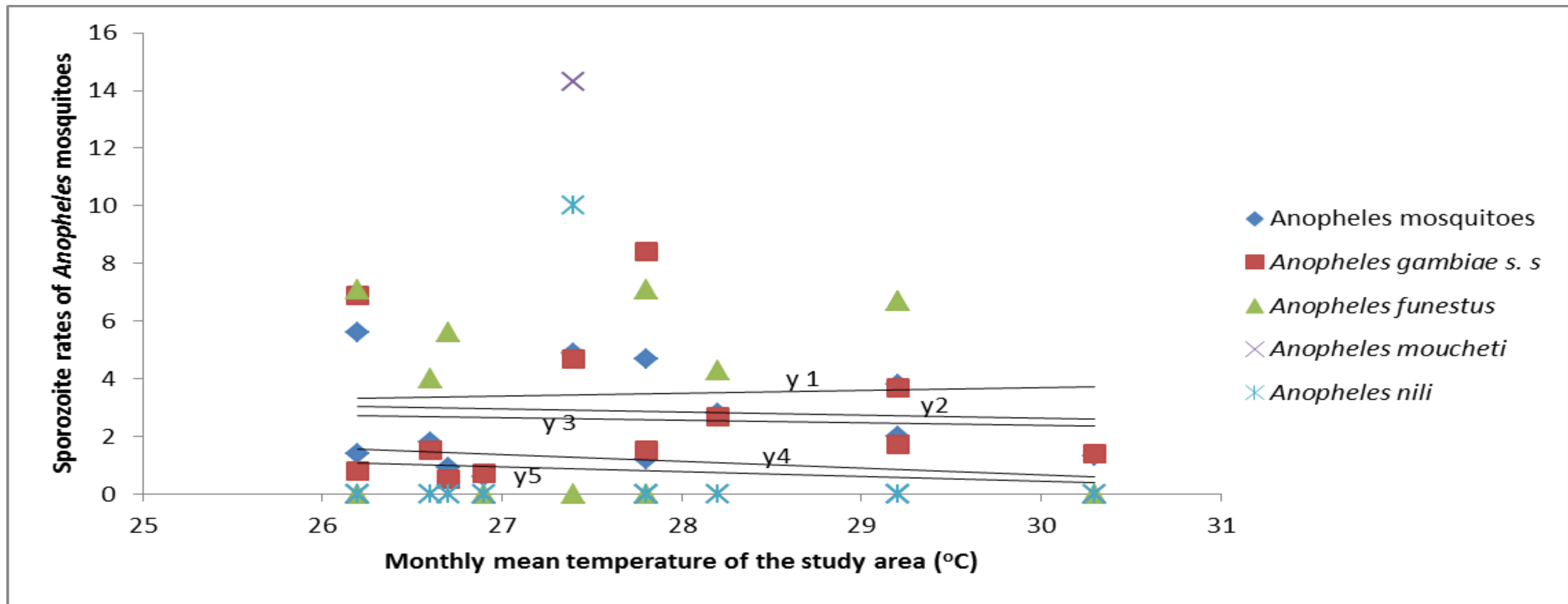


Figure 25: Relationship between temperature and sporozoite rates of *Anopheles* mosquitoes

From top to down: 1. *Anopheles funestus*, 2. *Anopheles gambiae* s.s., 3. *Anopheles* mosquitoes, 4. *Anopheles moucheti* and 5. *Anopheles nili*

4.4.2.2 Effect of rainfall on the sporozoite rate of *Anopheles* mosquitoes

The effect of rainfall on the sporozoite rates of *Anopheles* mosquitoes is shown in Figure 26 below. There was no significant correlation between the monthly mean rainfall and sporozoite rates of *Anopheles* mosquitoes. However, the relationship between rainfall and sporozoite rates of *Anopheles* mosquitoes is given by the regression equation: Sporozoite rate = -0.0013 rainfall + 2.9482 (R^2 = 0.0388). The relationship between rainfall and sporozoite rates of *An. gambiae* s. s. is given by the regression equation: Sporozoite rate = -0.0022 rainfall + 3.528 (R^2 = 0.0566). The relationship between rainfall and sporozoite rates of *An. funestus* is given by the regression equation: Sporozoite rate = -0.0004 rainfall + 3.5815 (R^2 = 0.0013). The relationship between rainfall and sporozoite rates of *An. moucheti* is given by the regression equation: Sporozoite rate = -0.005 rainfall + 2.6537 (R^2 = 0.1102). The relationship between rainfall and sporozoite rates of *An. nili* is given by the regression equation: Sporozoite rate = -0.0035 rainfall + 1.8557 (R^2 = 0.1102). However, there was no significant correlation between rainfall and sporozoite rate of *Anopheles* mosquitoes (r = -0.197, P = 0.539), *An. gambiae* s. s. (r = -0.238, P = 0.456), *An. funestus* (r = -0.036, P = 0.911) *An. moucheti* (r = -0.332, P = 0.292) and *An. nili* (r = -0.332, P = 0.292).

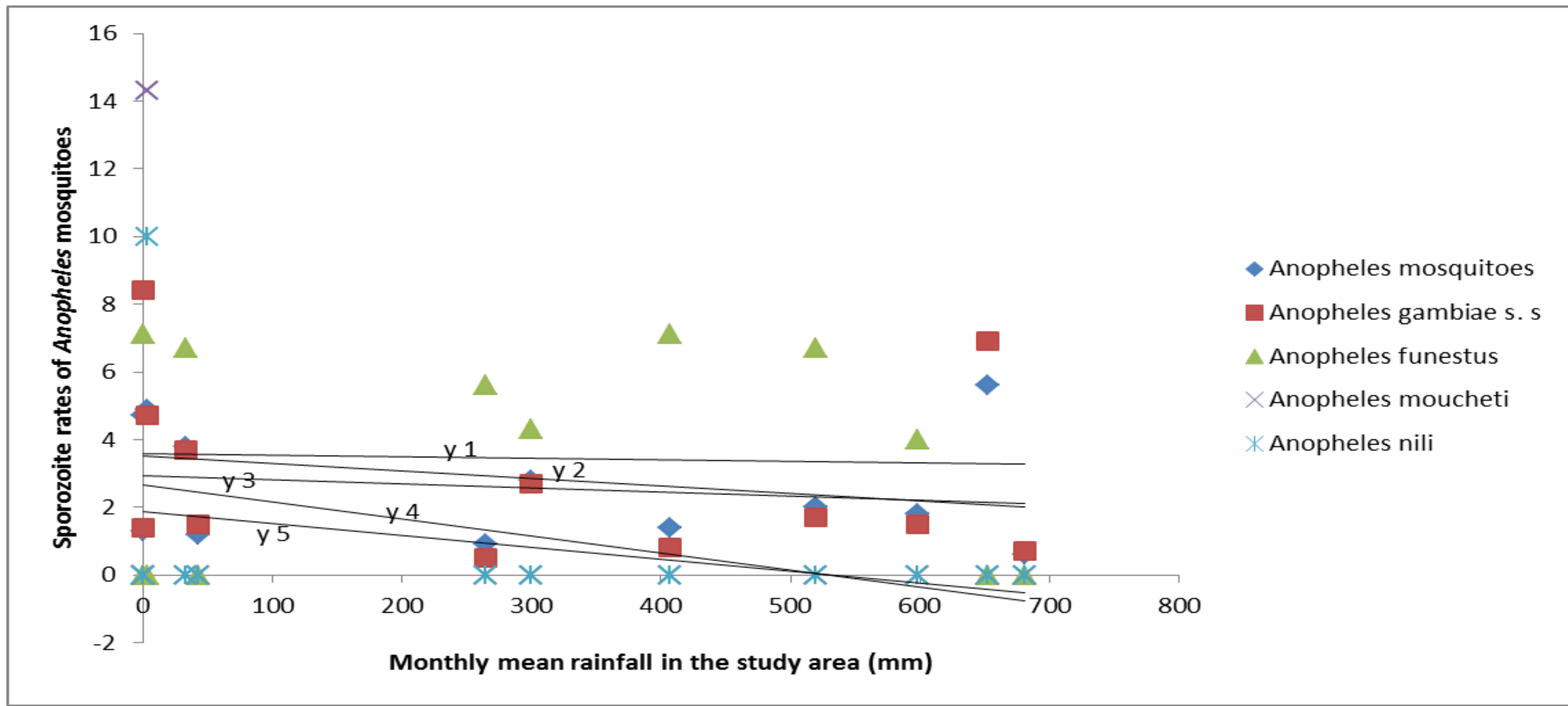


Figure 26: Relationship between rainfall and sporozoite rates of *Anopheles* mosquitoes.

From top to down: 1. *Anopheles funestus*, 2. *Anopheles gambiae* s. s., 3. *Anopheles* mosquitoes, 4. *Anopheles moucheti* and 5. *Anopheles nili*

4.4.2.3 Effect of relative humidity on the sporozoite rate of *Anopheles* mosquitoes

The effect of relative humidity on the sporozoite rates of *Anopheles* mosquitoes is shown in Figure 27 below. There was no significant correlation between the monthly mean relative humidity and sporozoite rates of *Anopheles* mosquitoes. However, the relationship between relative humidity and sporozoite rates of *Anopheles* mosquitoes is given by the regression equation: Sporozoite rate = -0.063 relative humidity + 7.5833 (R^2 = 0.0837). The relationship between relative humidity and sporozoite rates of *An. gambiae* s. s. is given by the regression equation: Sporozoite rate = -0.1013 relative humidity + 10.914 (R^2 = 0.0986). The relationship between relative humidity and sporozoite rates of *An. funestus* is given by the regression equation: Sporozoite rate = 0.0688 relative humidity – 1.9996 (R^2 = 0.0293). The relationship between relative humidity and sporozoite rates of *An. moucheti* is given by the regression equation: Sporozoite rate = -0.2115 relative humidity + 17.971 (R^2 = 0.1667). The relationship between relative humidity and sporozoite rates of *An. nili* is given by the regression equation: Sporozoite rate = -0.1479 relative humidity + 12.567 (R^2 = 0.1667). However, there was no significant correlation between relative humidity and sporozoite rate of *Anopheles* mosquitoes (r = -0.289, P = 0.362), *An. gambiae* s. s. (r = -0.314, P = 0.320), *An. funestus* (r = 0.171, P = 0.595) *An. moucheti* (r = -0.408, P = 0.188) and *An. nili* (r = -0.408, P = 0.188).

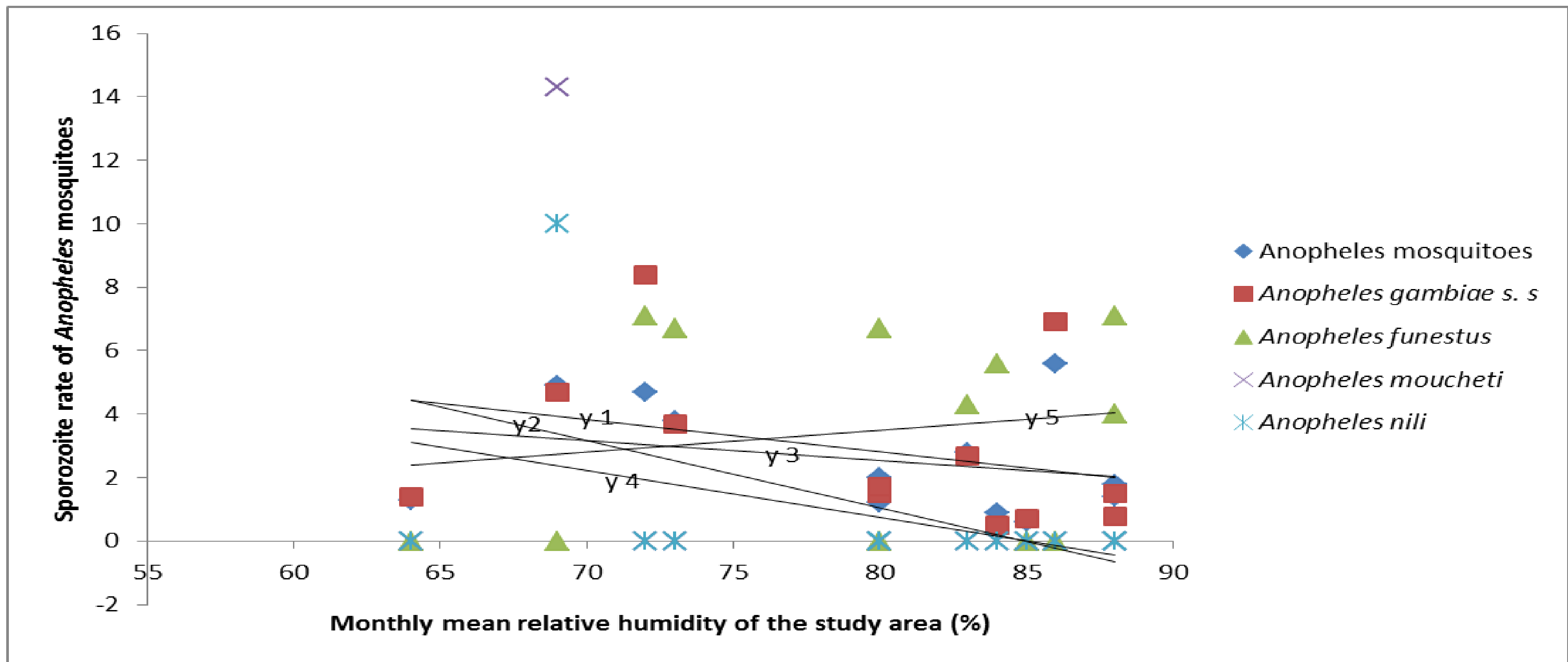


Figure 27: Relationship between relative humidity and sporozoite rates of *Anopheles* mosquitoes.

From top to down: 1. *Anopheles gambiae* s. s., 2. *Anopheles moucheti*, 3. *Anopheles* mosquitoes 4. *Anopheles nili* and, 5. *Anopheles funestus*.

4.4. 3 Entomological Innoculation Rate of *Anopheles* mosquitoes

The Entomological Innoculation rates of various blood fed *Anopheles* species collected in the study area are shown in Tables 24. The overall EIR of *Anopheles* mosquitoes in the study area was 0.123 bites/person/night.

An. gambiae s. s. recorded an overall EIR of 0.100 bites/person/night. *An. funestus* recorded an overall EIR of 0.021 bites/person/night. *An. moucheti* recorded an overall EIR of 0.002 bites/person/night. *An. nili* recorded an overall EIR of 0.004 bites/person/night. The difference in the EIR of the *Anopheles* species collected was statistically significant (P = 0.000; Appendix 92).

An. gambiae s. s. recorded a higher EIR of 0.102 bites/person/night in the dry season than in the wet season with EIR 0.074 bites/person/night (P = 0.327; Appendix 93). *An. funestus* recorded EIR of 0.014 bites/person/night for dry and 0.023 bites/person/night for wet seasons (P = 0.345; Appendix 94). *An. moucheti* recorded a higher EIR of 0.009 bites/person/night in the dry season than in the wet season with EIR 0.00 bites/person/night (P = 0.255; Appendix 95). *An. nili* recorded a higher EIR of 0.009 bites/person/night in the dry season than in the wet season with EIR 0.00 bites/person/night (P = 0.255; Appendix 96).

Table 24: Entomological Innoculation Rate (EIR) of *Anopheles* species collected from the study

Season	No. of Occupants (N)	Number of fed *Anopheles gambiae* s. s (F)	Man Biting Rate (M =F/N)	Sporozoite rate (S)	EIR (M × S)/100
Dry season	167	589	3.5	3.3	0.116
Wet season	241	1027	4.3	2.0	0.086
Total	408	1616	4.0	2.5	0.100
Season	No. of Occupants (N)	Number of fed *Anopheles funestus* (F)	Man Biting Rate (M =F/N)	Sporozoite rate (S)	EIR (M × S)/100
Dry season	167	86	0.5	2.8	0.014
Wet season	241	145	0.6	3.9	0.023
Total	408	231	0.6	3.5	0.021
Season	No. of Occupants (N)	Number of fed *Anopheles moucheti* (F)	Man Biting Rate (M =F/N)	Sporozoite rate (S)	EIR (M × S)/100
Dry season	167	29	0.2	4.3	0.009
Wet season	241	32	0.1	0.0	0.000
Total	408	61	0.1	2.0	0.002
Season	No. of Occupants (N)	Number of fed *Anopheles nili* (F)	Man Biting Rate (M =F/N)	Sporozoite rate (S)	EIR (M × S)/100
Dry season	167	28	0.2	4.5	0.009
Wet season	241	50	0.2	0.0	0.000
Total	408	78	0.2	1.5	0.003
Season	No. of Occupants (N)	Total Number of fed *Anopheles* mosquitoes (F)	Man Biting Rate (M =F/N)	Sporozoite rate (S)	EIR (M × S)/100
Dry season	167	732	4.4	3.3	0.145
Wet season	241	1254	5.2	2.1	0.109
Total	408	1986	4.9	2.5	0.123

4.5 Malaria endemicity through monthly and seasonal prevalence / intensity studies of malaria in the study area

4.5.1 Seasonal and Monthly prevalence of malaria

The seasonal and monthly prevalence of malaria in the study area are shown in Table 25. Out of 5400 blood specimen examined throughout the study period, a total of 3306 were positive for malaria parasite to give an overall prevalence of 61.2%. Wet season recorded a higher prevalence, 65.4% (2061/3150) of malaria parasite than the dry season with a prevalence record of 55.3% (1245). There was significant difference in the seasonal prevalence of malaria parasite (P = 0.000; Appendix 97). The month of April recorded the highest prevalence, 82.7% (372/450) of malaria parasite whereas the month of November recorded the least prevalence, 47.3% (213/450). There was significant difference in the monthly prevalence of malaria parasite throughout the study period (P = 0.000; Appendix 98). Within the dry season, the highest prevalence, 62.7% (282/450) of malaria parasite was recorded in the month of January and the least, 47.3% (213/450) in the month of November. There was significant difference in the monthly prevalence of malaria parasite during the dry season (P = 0.000; Appendix 99). Within the wet season, the highest prevalence, 82.7% (372/450) of malaria parasite was recorded in the month of April and the least, 54.7% (246/450) in the month of September. There was significant difference in the monthly prevalence of malaria parasite during the wet season (P = 0.000; Appendix 100).

Table 25: Seasonal and Monthly Malaria prevalence of malaria parasite

Seasonal prevalence				Monthly prevalence			
Season	**Number examined**	**Number positive**	**% positive**	**Month**	**Number examined**	**Number positive**	**% positive**
Dry season	2250	1245	55.3	November	450	213	47.3
				December	450	243	54.0
				January	450	282	62.7
				February	450	259	57.6
				March	450	248	55.1
Wet season	3150	2061	65.4	April	450	372	82.7
				May	450	300	66.7
				June	450	306	68.0
				July	450	315	70.0
				August	450	259	57.6
				September	450	246	54.7
				October	450	263	58.4
Total	**5400**	**3306**	**61.2**	**Total**	**5400**	**3306**	**61.2**

4.5.2 Prevalence of malaria parasite in the communities studied.

The prevalence of malaria parasite in the communities studied is shown in Table 26. Aguleri recorded an overall prevalence of 62.0% (1116/1800), dry season prevalence of 56.8% (426/750) and wet season prevalence of 65.7% (690/1050). Igbariam recorded an overall prevalence of 61.0% (1098/1800), dry season prevalence of 57.1% (428/750) and wet season prevalence of 63.8% (670/1050). Nsugbe recorded an overall prevalence of 60.7% (1092/1800), dry season prevalence of 52.1% (391/750) and wet season prevalence of 66.8% (701/1050). There was no significant difference in the prevalence of malaria parasite among the communities studied (P = 0.694; Appendix 101).

Table 26: Prevalence of malaria parasite infection in the communities studied

Months	No. examined per community	Aguleri		Igbariam		Nsugbe	
		No. Infected	Prevalence (%)	No. Infected	Prevalence (%)	No. Infected	Prevalence (%)
November	150	73	48.7	61	40.7	79	52.7
December	150	80	53.3	91	60.7	72	48.0
January	150	100	66.7	92	61.3	90	60.0
February	150	83	55.3	102	68.0	74	49.3
March	150	90	60.0	82	54.7	76	50.7
Total (dry season)	**750**	**426**	**56.8**	**428**	**57.1**	**391**	**52.1**
April	150	111	74.0	132	88.0	129	86.0
May	150	99	66.0	107	71.3	94	62.7
June	150	100	66.7	97	64.7	109	72.7
July	150	117	78.0	102	68.0	96	64.0
August	150	90	60.0	76	50.7	93	62.0
September	150	85	56.7	92	61.3	69	46.0
October	150	88	58.7	64	42.7	111	74.0
Total (wet season)	**1050**	**690**	**65.7**	**670**	**63.8**	**701**	**66.8**
Grand total	**1800**	**1116**	**62.0**	**1098**	**61.0**	**1092**	**60.7**

4.5.3 Prevalence of malaria parasite by gender

The prevalence of malaria parasite according to the gender of the study participants is shown in Table 27. The overall prevalence of 61.6% (1756/2852) in males is higher than that in females with 60.8% (2548/1550) prevalence value. In the dry season, males recorded a higher prevalence of 55.8% (656/1176) than females with 54.8% (589/1074) prevalence value. In the wet season, males also recorded a higher prevalence of 65.6% (1100/1676) than females with 54.8% (589/1074) prevalence value. However, the difference is not statistically significant (P = 0.578; Appendix 102).

Table 27: Prevalence of malaria parasite infection by gender

Months	Male			Female		
	No. examined	No. Infected	Prevalence (%)	No. examined	No. Infected	Prevalence (%)
November	234	111	47.4	216	102	47.2
December	241	133	55.2	209	110	52.6
January	239	150	62.8	211	132	62.6
February	235	137	58.3	215	122	56.7
March	227	125	55.1	223	123	55.2
Total (dry season)	**1176**	**656**	**55.8**	**1074**	**589**	**54.8**
April	242	200	82.6	208	172	82.7
May	244	163	66.8	206	137	66.5
June	244	165	67.6	206	141	68.4
July	243	171	70.4	207	144	69.6
August	240	138	57.5	210	121	57.6
September	225	124	55.1	225	122	54.2
October	238	139	58.4	212	124	58.5
Total (wet season)	**1676**	**1100**	**65.6**	**1474**	**961**	**65.2**
Grand total	**2852**	**1756**	**61.6**	**2548**	**1550**	**60.8**

4.5.4 Prevalence of malaria parasite by age

The prevalence of malaria parasite according to the age of the study participants is shown in Table 28. The overall prevalence of malaria parasite infection was highest in the age group 66 years and above with prevalence of 63.2% (283/448) and lowest in the age group 0 – 5 years with prevalence value of 56.5% (255/451). In the dry season, prevalence of malaria parasite infection was highest in the age group 66 years and above with prevalence of 60.6% (117/193) and lowest in the age group 36 – 45 years with prevalence value of 51.9% (164/316). In the wet season, prevalence of malaria parasite infection was highest in the age group 56 - 65 years with prevalence of 67.7% (224/331) and lowest in the age group 0 – 5 years with prevalence value of 59.2% (148/250). However, the difference in malaria parasite prevalence among various age groups is not statistically significant (P = 0.318; Appendix 103).

Table 28: Malaria parasite prevalence by Age

Month	0 – 5 years		6 – 15 years		16 – 25 years		26 – 35 years		36 – 45 years		46 – 55 years		56 – 65 years		66 years and above	
	E	P (%)	E	P (%)	E	P (%)	E	P (%)	E	P (%)	E	P (%)	E	P (%)	E	P (%)
November	44	43.2	55	43.6	86	41.9	76	47.4	60	56.7	42	52.4	46	52.2	41	43.9
December	41	63.4	54	59.3	86	58.1	76	47.4	64	46.9	44	52.3	45	46.7	40	62.5
January	45	44.4	54	57.4	85	63.5	77	64.9	63	66.7	43	67.4	47	70.2	36	63.9
February	35	60.0	56	62.5	88	63.6	78	55.1	64	48.4	43	48.8	46	50.0	40	72.5
March	36	58.3	53	50.9	87	63.2	81	59.3	65	41.5	45	44.4	47	59.6	36	61.1
Total (dry)	**201**	**53.2**	**272**	**54.8**	**432**	**58.1**	**388**	**54.9**	**316**	**51.9**	**217**	**53.0**	**231**	**55.8**	**193**	**60.6**
April	36	77.8	54	75.9	87	82.8	80	81.3	68	77.9	43	90.7	47	87.2	35	94.3
May	38	60.5	52	65.4	87	66.7	77	64.9	62	67.7	46	63.0	47	72.3	41	73.2
June	41	53.7	54	63.0	85	64.7	77	70.1	65	81.5	47	70.2	45	73.3	36	61.1
July	31	58.1	50	68.0	87	73.6	81	71.6	70	65.7	46	71.7	49	69.4	36	77.8
August	36	58.3	54	51.9	87	63.2	80	61.3	64	43.8	45	51.1	48	68.8	36	61.1
September	23	47.8	44	52.3	85	52.9	87	60.9	76	60.5	48	60.4	56	53.6	31	29.0
October	45	44.4	60	60.0	87	58.6	73	57.5	63	63.5	43	65.1	39	48.7	40	55.0
Total (wet)	**250**	**59.2**	**368**	**62.5**	**605**	**66.1**	**555**	**66.8**	**468**	**65.8**	**318**	**67.3**	**331**	**67.7**	**255**	**65.1**
Grand Total	**451**	**56.5**	**640**	**59.2**	**1037**	**62.8**	**943**	**61.9**	**784**	**60.2**	**535**	**61.5**	**562**	**62.8**	**448**	**63.2**

Key: E means Number examined, P means Prevalence

4.5.5 Relationship between malaria parasite prevalence and sporozoite rate of *Anopheles species*.

The relationship between malaria parasite prevalence and sporozoite rate of *Anopheles species* is shown in Figure 28. The relationship between malaria parasite prevalence and sporozoite rate of all the *Anopheles* mosquitoes is given by the regression equation: Sporozoite rate = 0.00209 Malaria prevalence + 1.3021 ($R^2 = 0.0129$). The relationship between malaria parasite prevalence and sporozoite rate of *Anopheles gambiae* s. s. is given by the regression equation: Sporozoite rate = -0.0002 Malaria prevalence + 2.8869 ($R^2 = 0.000001$). The relationship between malaria parasite prevalence and sporozoite rate of *Anopheles funestus* is given by the regression equation: Sporozoite rate = 0.0073 Malaria prevalence + 3.014 ($R^2 = 0.0005$). The relationship between malaria parasite prevalence and sporozoite rate of *Anopheles moucheti* is given by the regression equation: Sporozoite rate = 0.0215 Malaria prevalence – 0.1233 ($R^2 = 0.0024$). The relationship between malaria parasite prevalence and sporozoite rate of *Anopheles nili* is given by the regression equation: Sporozoite rate = 0.015 Malaria prevalence - 0.0862 ($R^2 = 0.0024$). However, there was no significant correlation between malaria parasite prevalence and sporozoite rates of *Anopheles* mosquitoes ($r = 0.114$, $P = 0.725$), *Anopheles gambiae* s. s. ($r = -0.001$, $P = 0.998$), *Anopheles funestus* ($r = 0.021$, $P = 0.948$), *Anopheles moucheti* ($r = 0.049$, $P = 0.880$) and *Anopheles nili* ($r = 0.049$, $P = 0.880$).

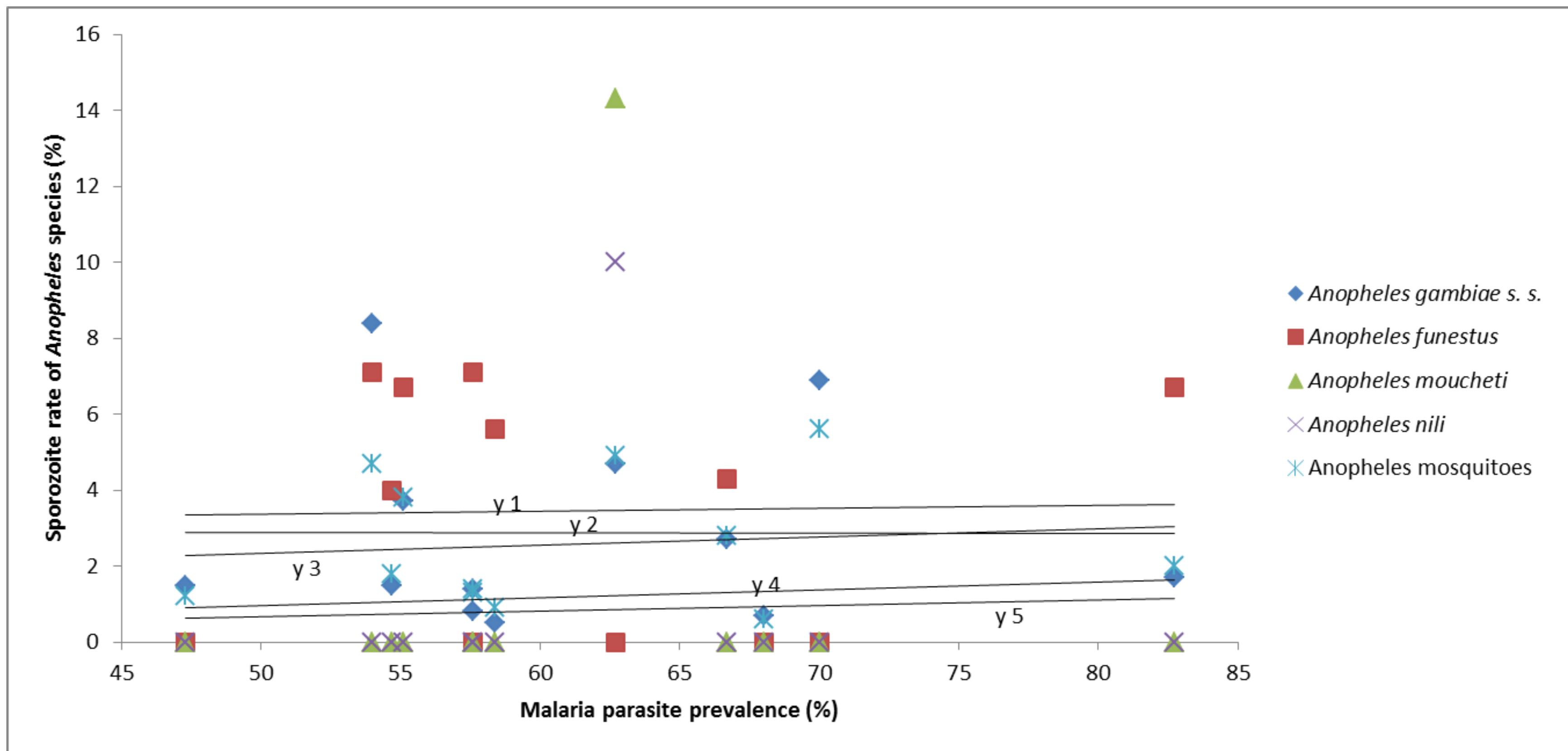


Figure 28: Relationship between the prevalence of malaria parasite and sporozoite rates of *Anopheles* mosquitoes

From top to down: 1. *Anopheles funestus*, 2.. *Anopheles gambiae* s. s., 3. *Anopheles* mosquitoes, 4. *Anopheles moucheti* and 5. *Anopheles nili*

4.5.6 Seasonal and monthly intensity of Malaria parasite.

Seasonal and Monthly mean intensity of malaria parasite infection is shown in Table 29. The overall mean malaria parasite intensity for the 3306 specimen positive for malaria parasites is 263.4±3.2 malaria parasites / µl of blood. Dry season mean intensity of 298.5±5.2 was higher than the wet season mean intensity of 242±4.0 malaria parasites / µl of blood. There was significant difference in the seasonal intensity of malaria parasite (P = 0.000; Appendix 104). The month of October recorded the highest mean intensity of 332.3±12.1 malaria parasites / µl of blood whereas the month of July recorded the least mean intensity of 209.8±8.7 malaria parasites / µl of blood. There was significant difference in the monthly intensity of malaria parasite throughout the study period (P = 0.000; Appendix 105). Within the dry season, the highest mean intensity of 327.9±13.0 malaria parasites / µl of blood was recorded in the month of November and the least mean intensity of 269.5±10.8 malaria parasites / µl of blood was recorded in the month of February. There was significant difference in the monthly intensity of malaria parasite during the dry season (P = 0.005; Appendix 106). Within the wet season, the highest mean intensity of 332.3±12.1 malaria parasites / µl of blood was recorded in the month of October and the least mean intensity of 209.8±8.7malaria parasites / µl of blood was recorded in the month of July. There was significant difference in the monthly intensity of malaria parasite during the wet season (P = 0.000; Appendix 107).

Table 29: Seasonal and Monthly mean intensity of malaria parasite infection

Seasonal intensity			Monthly intensity		
Season	**Number positive**	**Mean intensity±se**	**Month**	**Number positive**	**Mean intensity±se**
Dry season	1245	298.5±5.2	November	213	327.9±13.0
			December	243	310.9±12.0
			January	282	304.3±10.5
			February	259	269.5±10.8
			March	248	284.6±11.6
Wet season	2061	242±4.0	April	372	269.0±9.6
			May	300	232.2±9.9
			June	306	214.4±8.6
			July	315	209.8±8.7
			August	259	221.6±11.6
			September	246	215.6±13.4
			October	263	332.3±12.1
Total	**3306**	**263.4±3.2**	**Total**	**3306**	**263.4±3.2**

4.5.7 Malaria parasite intensity in the communities

The intensity of malaria parasite infection in various communities studied is shown in Table 30. The intensity values obtained were 261±5.7 malaria parasites / µl of blood in Aguleri, 261±5.5 malaria parasites / µl of blood in Igbariam and 267±5.5 malaria parasites / µl of blood at Nsugbe. There was no significant difference in malaria parasite intensity among the communities (P = 0.686; Appendix 108).

Table 30: Malaria parasite intensity in the communities

Months	Aguleri		Igbariam		Nsugbe	
	No. Infected	**Mean Intensity±SE**	**No. Infected**	**Mean Intensity±SE**	**No. Infected**	**Mean Intensity±SE**
November	73	328.8±21.9	61	344.9±24.3	79	313.9±22.0
December	80	317.5±21.6	91	323.7±20.3	72	287.2±20.3
January	100	313.2±18.6	92	308.4±18.8	90	290.2±17.2
February	83	271.3±19.7	102	268.2±17.6	74	269.2±19.2
March	90	273.3±20.0	82	280.6±18.9	76	302.4±21.6
Total (dry season)	**426**	**300.1±9.1**	**428**	**302.0±8.9**	**391**	**292.8±8.9**
April	111	292.3±18.0	132	248.2±15.2	129	270.4±16.8
May	99	225.5±17.5	107	231.4±16.7	94	240.2±17.8
June	100	197.6±14.6	97	211.5±15.4	109	232.3±14.7
July	117	197.6±14.2	102	216.3±15.9	96	217.9±15.1
August	90	225.3±20.7	76	208.4±17.9	93	228.8±21.0
September	85	194.4±22.6	92	219.1±21.2	69	237.1±26.8
October	88	330.0±20.9	64	347.5±26.1	111	325.4±18.1
Total (wet season)	**690**	**236.9±7.1**	**670**	**236.3±6.9**	**701**	**253.2±7.0**
Grand total	**1116**	**261.0±5.7**	**1098**	**261.9±5.5**	**1092**	**267.4±5.5**

4.5.8 Malaria parasite intensity according to gender

The intensity of malaria parasite infection according to the gender of the study participants is shown in Table 31. The overall mean intensity of 266.3±4.5 malaria parasites / µl of blood in males is higher than the mean intensity of 260.2±4.6 malaria parasites / µl of blood in females. In the dry season, males recorded a higher mean intensity of 300.2±7.1 malaria parasites / µl of blood than females with mean intensity of 296.6±7.5 malaria parasites / µl of blood. However, in the wet season, females recorded a higher mean intensity of 331.6±17.4 malaria parasites / µl of blood than males with mean intensity of 246.1±5.6 malaria parasites / µl of blood. However, the difference in malaria parasite intensity between males and females is not statistically significant (P = 0.341; Appendix 109).

Table 31: Malaria parasite intensity by gender

Months	Male		Female	
	No. Infected	**Mean Intensity±SE**	**No. Infected**	**Mean Intensity±SE**
November	111	304.1±18.1	102	353.7±18.5
December	133	324.8±16.6	110	294.0±17.3
January	150	309.3±14.5	132	298.6±15.4
February	137	272.1±15.1	122	266.6±15.4
March	125	290.1±15.8	123	279.1±17.1
Total (dry season)	**656**	**300.2±7.1**	**589**	**296.6±7.5**
April	200	273.2±13.5	172	264.2±13.6
May	163	229.6±14.0	137	235.3±14.0
June	165	219.9±11.5	141	207.9±12.9
July	171	209.2±12.5	144	210.6±11.8
August	138	226.4±16.5	121	216.2±16.4
September	124	234.5±19.3	122	196.4±18.5
October	139	332.9±16.8	124	331.6±17.4
Total (wet season)	**1100**	**246.1±5.6**	**961**	**331.6±17.4**
Grand total	**1756**	**266.3±4.5**	**1550**	**260.2±4.6**

4.5.9 Malaria parasite intensity according to age

The intensity of malaria parasite infection according to the age of the study participants is shown in Table 32. The overall mean intensity of malaria parasite infection was highest in the age group 16 – 25 years with 274.3±7.4 malaria parasites / µl of blood and lowest in the age group 26 – 35 years with mean intensity of 254.1±7.8 malaria parasites / µl of blood. In the dry season, the mean intensity of malaria parasite infection was highest in the age group 36 – 45 years with 327.4±14.7 malaria parasites / µl of blood and lowest in the age group 26 – 35 years with mean intensity of 282.9±13.3 malaria parasites / µl of blood. In the wet season, the mean intensity of malaria parasite infection was highest in the age group 16 – 25 years with 261.6±9.6 malaria parasites / µl of blood and lowest in the age group 56 – 65 years with mean intensity of 225.9±12.0 malaria parasites / µl of blood However, the difference in the mean intensity of malaria parasite among various age groups is not statistically significant (P = 0.691; Appendix 110).

Table 32: Malaria parasite intensity by Age

Month	0 – 5 years	6 – 15 years	16 – 25 years	26 – 35 years	36 – 45 years	46 – 55 years	56 – 65 years	66 years and above
November	290.5±38.2	291.7±28.3	320.0±32.0	322.2±34.6	401.2±32.1	327.3±47.8	385.0±38.7	228.9±36.4
December	258.5±32.5	322.5±31.1	361.6±26.1	281.1±30.4	302.0±38.1	274.8±29.6	320.0±48.0	328.0±41.8
January	308.0±42.4	291.6±26.8	301.5±24.5	288.2±27.0	364.8±27.1	311.7±31.9	255.8±30.5	309.6±34.1
February	310.5±40.9	268.6±27.0	250.0±20.5	215.8±26.6	283.9±34.6	299.0±44.2	347.8±29.8	259.3±34.2
March	266.7±39.6	248.9±33.7	255.5±23.7	309.2±29.3	254.8±26.7	318.0±43.1	315.7±37.8	331.8±39.7
Total (dry)	**285.2±17.0**	**285.1±13.2**	**294.5±11.4**	**282.9±13.3**	**327.4±14.7**	**306.1±17.2**	**319.7±16.6**	**292.8±16.9**
April	265.0±37.5	291.7±30.8	314.4±22.0	268.6±22.1	214.3±24.3	246.2±25.3	271.2±29.3	258.2±34.2
May	248.7±40.8	191.8±28.5	237.2±24.5	229.6±26.9	201.0±19.1	283.4±28.7	231.8±30.3	254.7±31.0
June	252.7±33.0	256.5±28.5	195.6±19.4	219.3±20.8	220.4±21.2	166.1±22.6	198.8±23.5	227.3±35.3
July	206.7±37.9	204.1±26.2	215.0±17.0	192.4±20.0	228.7±22.7	208.5±31.5	225.9±32.1	194.3±22.7
August	230.5±38.6	198.6±33.3	238.5±26.4	211.4±26.6	224.3±41.6	276.5±41.8	201.2±28.4	192.7±38.4
September	214.5±62.7	224.3±40.5	240.0±37.5	230.9±28.5	241.7±33.8	187.6±32.4	165.3±34.0	106.7±30.6
October	355.2±35.9	306.7±33.2	388.2±25.2	323.8±33.4	310.0±31.9	320.0±35.3	303.2±50.2	316.4±40.2
Total (wet)	**260.1±15.2**	**243.0±12.1**	**261.6±9.6**	**237.6±9.6**	**233.1±10.4**	**238.0±11.9**	**225.9±12.0**	**233.5±13.4**
Grand Total	**270.7±11.3**	**259.6±9.0**	**274.3±7.4**	**254.1±7.8**	**265.9±8.7**	**261.8±9.9**	**260.2±10.0**	**258.0±10.7**

4.5.10 The relationship between malaria parasite intensity and sporozoite rate

Figure 29 shows the relationship between malaria parasite intensity and sporozoite rate. The relationship between malaria parasite intensity and sporozoite rate of all the *Anopheles* mosquitoes is given by the regression equation: Sporozoite rate = 0.0009 Malaria intensity + 2.3527 ($R^2 = 0.0005$). The relationship between malaria parasite intensity and sporozoite rate of *Anopheles gambiae* s. s. is given by the regression equation: Sporozoite rate = 0.0057 Malaria intensity + 1.3455 ($R^2 = 0.0107$). The relationship between malaria parasite intensity and sporozoite rate of *Anopheles funestus* is given by the regression equation: Sporozoite rate = 0.0073 Malaria intensity + 1.5127 ($R^2 = 0.0112$). The relationship between malaria parasite intensity and sporozoite rate of *Anopheles moucheti* is given by the regression equation: Sporozoite rate = 0.0203Malaria intensity – 4.9928 ($R^2 = 0.0679$). The relationship between malaria parasite intensity and sporozoite rate of *Anopheles nili* is given by the regression equation: Sporozoite rate = 0.0163 Malaria intensity – 3.4915 ($R^2 = 0.0679$). However, there was no significant correlation between malaria parasite intensity and sporozoite rates of *Anopheles* mosquitoes ($r = 0.023$, $P = 0.943$), *Anopheles gambiae* s. s. ($r = 0.103$, $P = 0.749$), *Anopheles funestus* ($r = 0.106$, $P = 0.744$) *Anopheles moucheti* ($r = 0.261$, $P = 0.413$) and *Anopheles nili* ($r = 0.261$, $P = 0.413$).

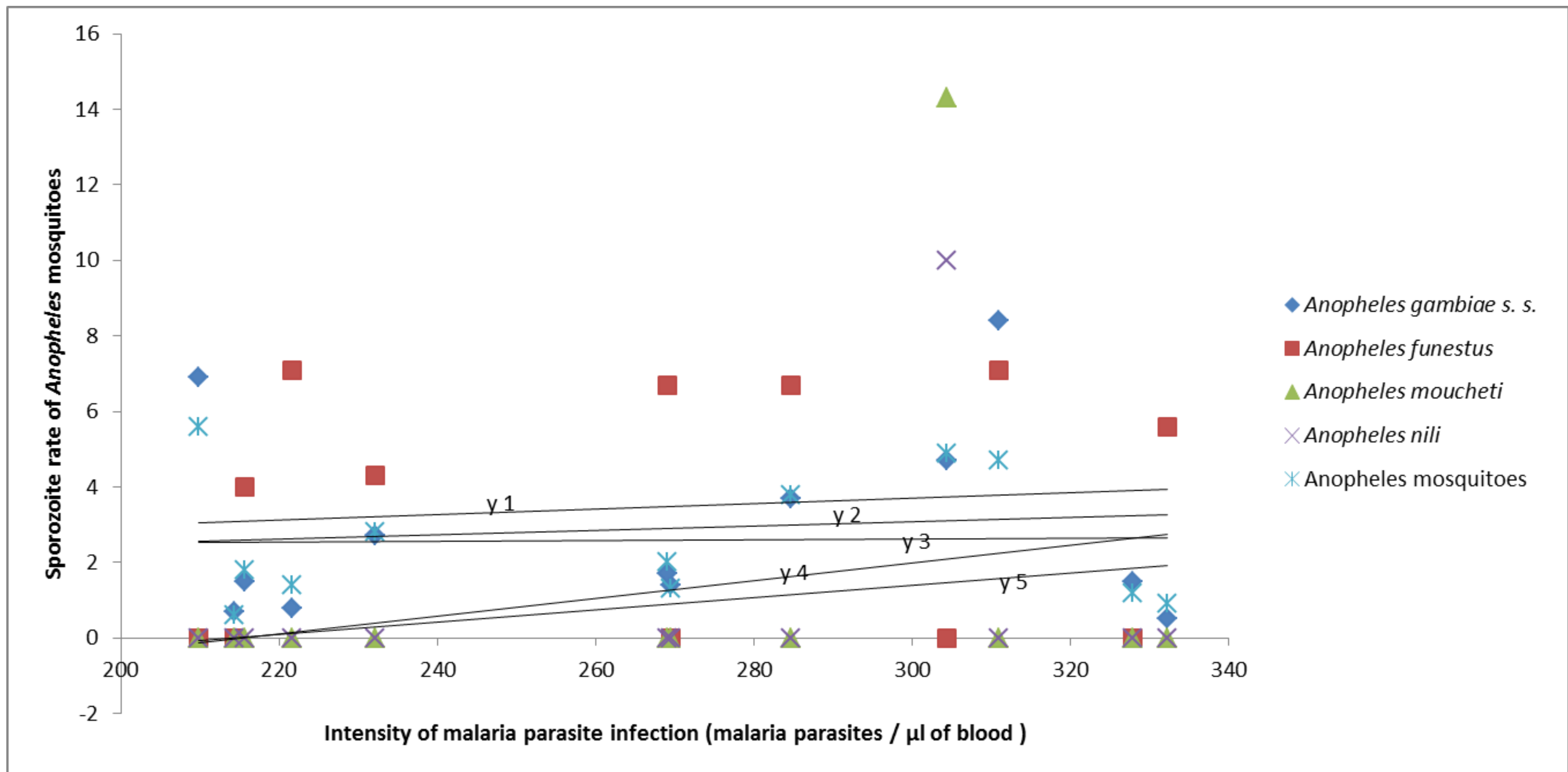


Figure 29: Relationship between the mean malaria parasite intensity and sporozoite rates of *Anopheles* mosquitoes.

From top to down: 1. *Anopheles funestus* 2. *Anopheles gambiae* s. s., 3. *Anopheles* mosquitoes 4. *Anopheles moucheti* 5. *Anopheles nili*,

4.5.11 Effect of temperature on the prevalence and intensity of malaria parasite infection

The effect of temperature on the prevalence and intensity of malaria parasite infection is shown in Figure 30 below. There was no significant correlation between the monthly mean temperature and malaria parasite prevalence ($r = 0.078$, $P = 0.809$) and intensity ($r = 0.307$, $P = 0.332$). Nevertheless, the relationship between the environmental temperature and prevalence of malaria parasite infection is given by the regression equation: Prevalence = 0.5607 Temperature + 45.696 ($R^2 = 0.0061$). The relationship between the environmental temperature and intensity of malaria parasite infection is given by the regression equation: Intensity = 10.813 Temperature – 33.595 ($R^2 = 0.0942$).

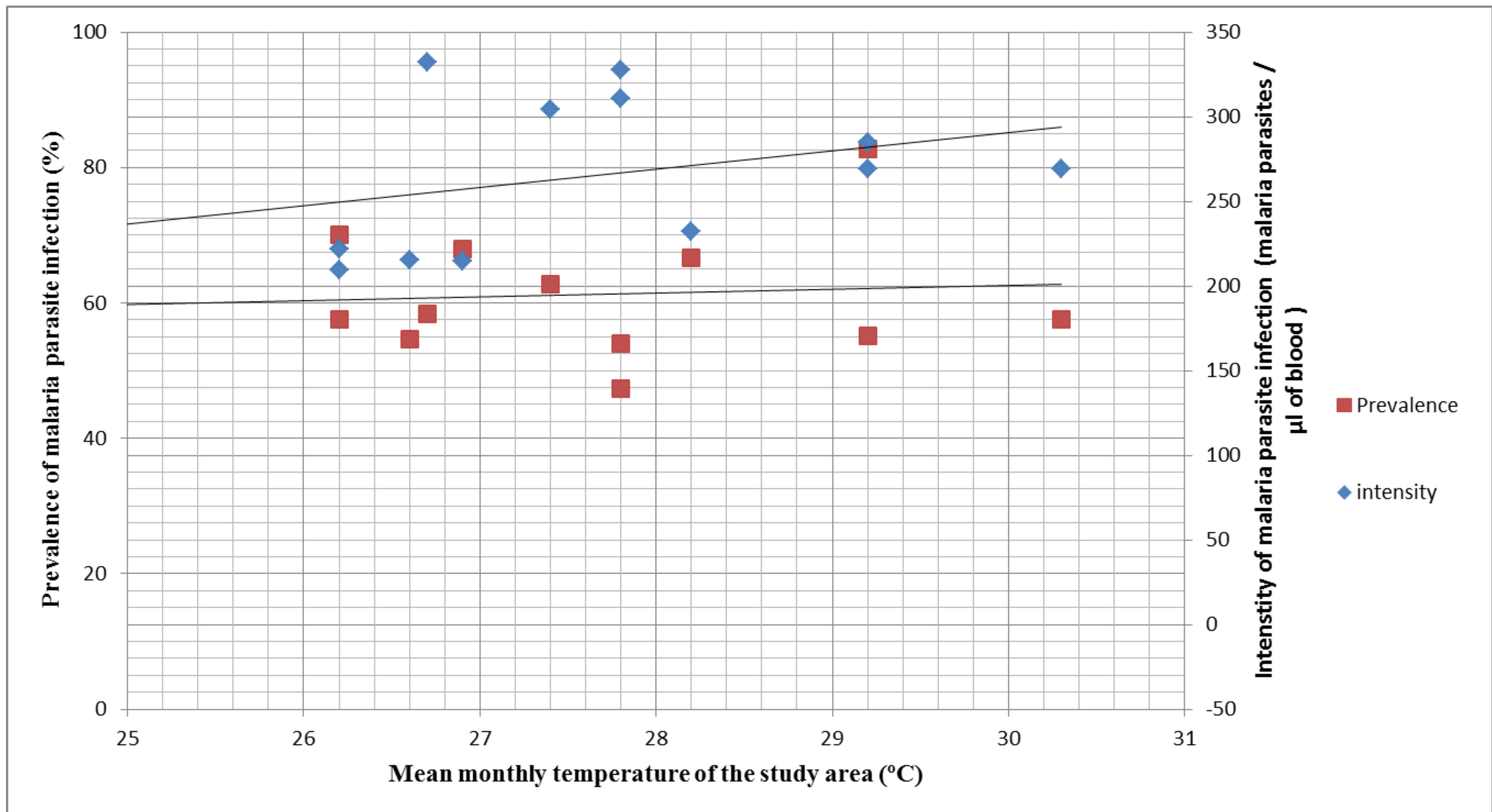


Figure 30: Effect of temperature on the prevalence and intensity of malaria parasite infection

4.5.12 Effect of rainfall on the prevalence and intensity of malaria parasite infection

The effect of rainfall on the prevalence and intensity of malaria parasite infection is shown in Figure 31 below. There was no significant correlation between the monthly mean rainfall and malaria parasite prevalence ($r = 0.571$, $P = 0.053$). However, there was significant strong negative correlation between the monthly mean rainfall and malaria parasite intensity ($r = -0.773$, $P = 0.003$). The relationship between rainfall and prevalence of malaria parasite infection is given by the regression equation: Prevalence = 0.0197 Rainfall + 55.496 ($R^2 = 0.3258$). The relationship between rainfall and intensity of malaria parasite infection is given by the regression equation: Intensity = -0.1307 Rainfall + 304.16 ($R^2 = 0.5975$).

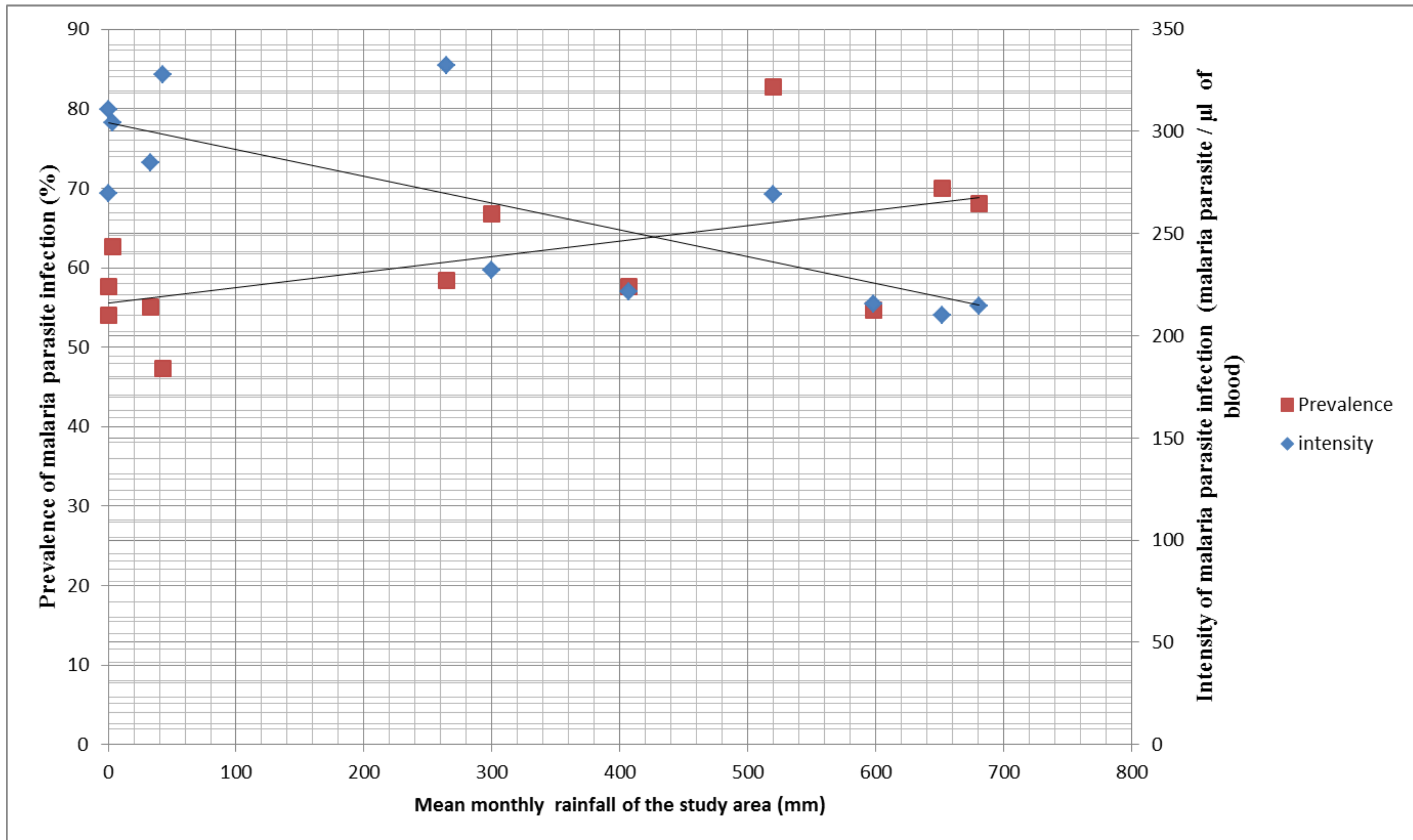


Figure 31: Effect of rainfall on the prevalence and intensity of malaria parasite infection.

4.5.13 Effect of relative humidity on the prevalence and intensity of malaria parasite infection

The effect of relative humidity on the prevalence and intensity of malaria parasite infection is shown in Figure 32 below. There was no significant correlation between relative humidity and malaria parasite prevalence ($r = 0.183$, $P = 0.564$) and intensity ($r = -0.0532$, $P = 0.075$). However, the relationship between relative humidity and prevalence of malaria parasite infection is given by the regression equation: Prevalence = 0.2189 Relative humidity + 43.864 ($R^2 = 0.0343$). The relationship between relative humidity and intensity of malaria parasite infection is given by the regression equation: Intensity = -3.0865 Relative humidity + 510.87 ($R^2 = 0.2826$).

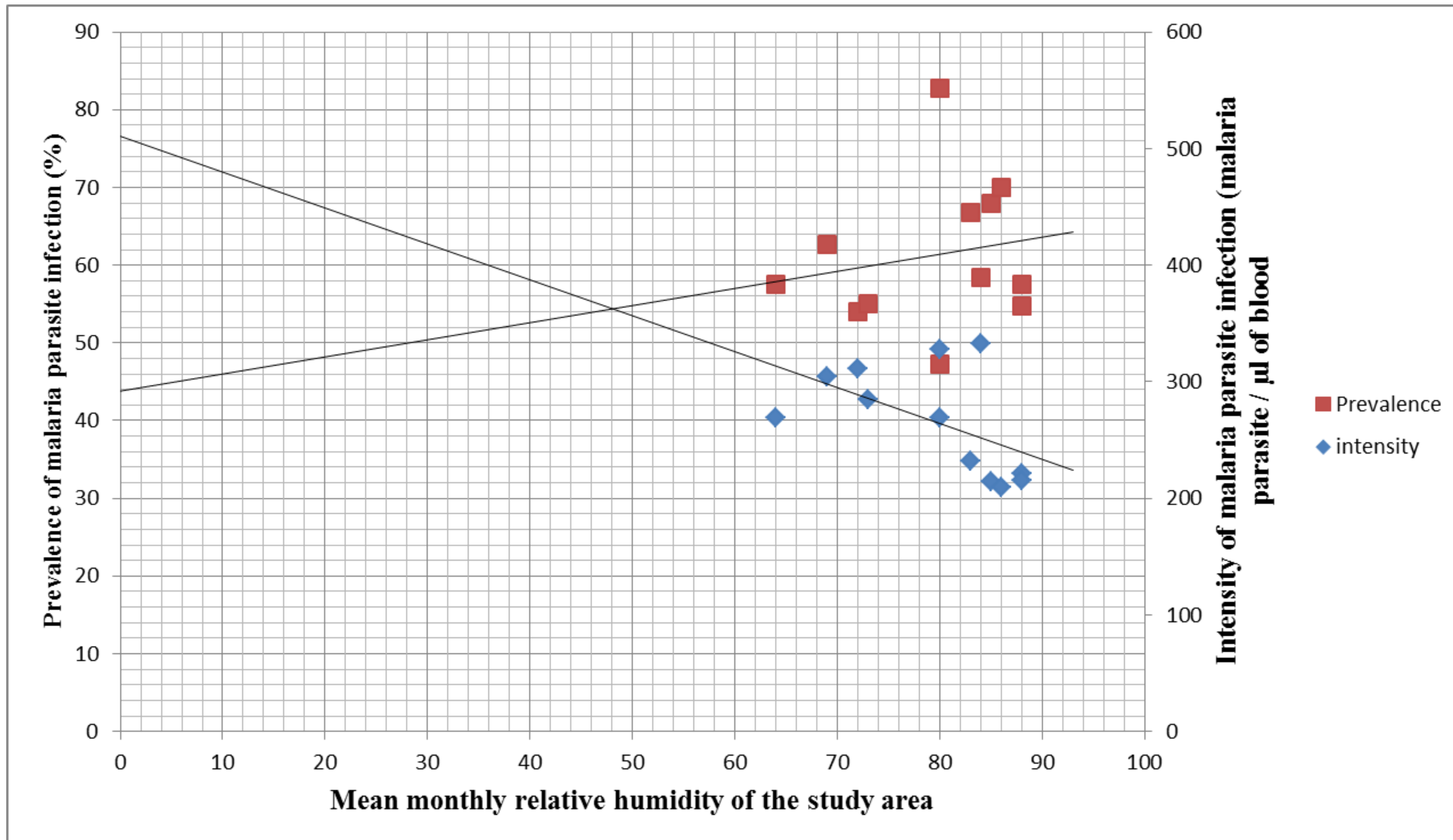


Figure 32: Effect of relative humidity on the prevalence and intensity of malaria parasite infection

CHAPTER FIVE

DISCUSSION

5.1 Malaria vector species composition and the climatic factors influencing their survival and population.

The study recorded the occurrence and high abundance of *Anopheles* mosquitoes in the study area. *An. gambiae* s. l., *An. funestus, An. moucheti and An. nili* were the vectors of malaria parasite identified. Within the *Anopheles g*ambiae s. l., *Anopheles gambiae s. s.* was the only member of the complex identified in the study area. This was the same in each of the communities surveyed within the study area; where *An. gambiae* s. s., *An. funestus, An. moucheti* and *An. nili* were all identified. This gave 100% *Anopheles* species overlap among the communities. The abundance of each of the *Anopheles* species in each of the communities is also similar. This is because the communities are within the same ecological locale and have similar climatic and environmental conditions.

The result of this present work is partially different from the findings of Labbo *et al*. (2016), Coetzee *et al*. (2013), Battle *et al*. (2012), Sinka *et al*. (2012), and Okorie *et al.* (2011) who reported *An. arabiensis* and *An. coluzzii,* alongside *An. gambiae* s. s. as the dominant member of *An. gambiae* s. l. in Africa. In the same vein, Dzorgbe *et al*. (2017) reported both *An. coluzzii* and *An. gambiae s. s* as the most abundant sibling species of *An. gambiae* s. l. identified in Ghana. *An. melas* and *An. merus* have also been reported in some areas in Africa (Ebenezar *et al*., 2016; Kipyab *et al*., 2013; Oyewole *et al.* 2010; Mendis *et al*., 2000 and Cuamba and Mendis, 2009). This shows that there is large variation in the spatial distribution of members of *An. gambiae* s. l.

Apart from *An. gambiae* s. s, other *Anopheles* species (*An. funestus*, *An. nili* and *An. moucheti*) were also reported in the present study area. This is line with the finding of Okorie *et al*., (2011); Molineaux and Gramiccia, (1980); Gilles and Coetzee, (1987) who reported them as common species of mosquitoes that transmit malaria parasites in Nigeria. However, the species identified in the study did not include *An. rufipes* and *An. coustani* reported by Mattah *et al*., (2017). The

disparity between *Anopheles* species composition of the study area and other areas in Africa could be due to climatic and environmental differences found within the region.

The *Anopheles* species identified in the study were collected both as larvae and as adults without any significant difference in the population of the two lifecycle stages. It shows that they are local populations of *Anopheles* species found in the study area; being able to breed and survive in the area. The dorminant species was *An. gambiae* s. s. and this was followed by *An. funestus*. *An. nili* and *An. moucheti* occurred at the same level.

These *Anopheles* species were found all through the year with varied monthly peaks. This is because the mosquito vectors may not be distributed homogenously both in space and time. The abundance of *An. gambiae* s. s peaked in the months of May, *An. funestus* in the month of March, *An. moucheti* in the month of June and *An. nili* in the month of April. There was also seasonal variability shown by only *An. gambiae* s. s. which recorded higher abundance during the wet season. This is in line with the report of Okullo *et al.* (2017) who stated that population density of *Anopheles* species increased tremendously between May and June and that corresponds to the peak of rain. The variation in the seasonal abundance of *An. gambiae* s. s. is attributed to the difference in the amount of rainfall during the seasons. This is because there was strong positive correlation between the amount of rainfall and the abundance of *Anopheles* species. When the amount of rainfall increases, the abundance of *An. gambiae* s. s increases and vice versa. This is similar to the findings of Simon-Oke and Olofintoye, (2015) who also reported that *An. gambiae* s. s. had the highest abundance during the rainy periods. There were no significant relationships between *Anopheles* species abundance and temperature as well as with relative humidity. This implies that the *Anopheles* species are well adapted to the temperature range of 26.2ºC – 30.3ºC and relative humidity range of 64% – 88% found in the study area. This further emphasizes that rainfall is the main climatic factor that cause temporal change in *Anopheles* species population, particularly *An. gambiae* s. s. because more breeding sites are created during the period of rainy season.

5.2 Breeding ecology, physicochemical and biological factors operating in *Anopheles* mosquito habitat.

Anopheles mosquitoes were found to breed in the following breeding habitats within the study area: basin, clay pots, canoes, drainage channels, head pans, plastic drums/containers, puddles, river banks and swamps. It shows that different sizes of both natural and artificial water collections provide suitable breeding sites for *Anopheles* species in the study area. This is supported by the findings of Adeleke *et al*., (2008), Ndenga *et al*., (2011) and Onyido *et al.* (2014). All these breeding sites fall into the category of open larval habitats that have large exposure to sunlight. This is in line with the well known fact that *Anopheles* species breed in in sunlit stagnant water collections around our homes, streets, and streams or other quiescent water collections (Onyido *et al*., 2014; Robert and Janovay, 2010). It was also evident in the study that the *Anopheles* species encountered used multiple breeding sites for oviposition; a finding similar to that of Chen *et al*. (2006).

Both *An. gambiae* s. s. and *An. funestus* larvae were found to utilize all the identified *Anopheles* mosquito breeding habitats. *An. moucheti* and *An. nili* were absent in the following breeding sites: basin, head pans, and puddles. It appears that *An. gambiae* s. s. and *An. funestus* utilize exactly the same breeding habitats; similarly, *An. moucheti* and *An. nili* utilize exactly the same breeding habitats. There is similarity in the distribution of the breeding sites within the communities studied. This is because the communities are all riverine, in addition to the presence of other manmade habitats that could be obtained in any other location.

Availability and stability of *Anopheles* species breeding habitats is very crucial in determining year round productivity of malaria vectors (Himeidan *et al*., 2009). Apart from head pans, other breeding sites were available in all the months within the wet season. Canoes, plastic drums / containers and river banks, were also available in all the months within the dry season. This simply means that Bank of rivers, canoe and water storage containers like plastic drums are the most stable breeding habitats encountered in the study area. The rivers constitute the natural habitats whereas broken plastic containers, canoes, and plastic drums are manmade habitats. The natural habitats are available all year round. The manmade habitats are also available all years round because they are utilized by man regularly for water storage. Canoe as a manmade habitat

is always used in water bodies in the study area for transportation and can retain some quantity of water that constitutes breeding site for *Anopheles* species. Dugout canoes that are unused for days can be partially filled with rainwater or water that seeps through the joints between woods at the base of the canoe to become mosquito nurseries. Other breeding sites encountered in the study area were present only at certain months of the year especially during the wet season. It is either that they dry up or are removed during environmental sanitation.

Even among the available breeding habitats, malaria vectors are often present, abundant and their adults produced in large numbers in some habitats and not in others. *An. gambiae* s. s. was found to breed in basins, clay pot, canoe, drainage channels, bank of river, head pans, plastic drums / containers, puddles and swamps. Nevertheless, *An. gambiae* s. s. was present and more abundant in some breeding sites and not in others. River bank was generally the most productive habitat for *An. gambiae* s. s. Within late wet season and the dry season, *An. gambiae* s. s. breeding was sustained by river banks. At the peak of rainy season, drainage channels and clay pot sustained the breeding of *An. gambiae* s. s. This is similar to the report of Onyido *et al*., (2014) that *An. gambiae* s. s larvae are occasionally found in man-made containers such as wheel barrows, mortar, pans, open tanks, canoes and abandoned concrete mixers.

An. funestus was found to breed in basin, clay pots, canoe, drainage channels, river banks, head pan, plastic drum / container, puddles and swamps. Nevertheless, *An. funestus* were present and more abundant in some breeding sites and not in others. River bank was generally the most productive habitat for *An. funestus*. In the rainy season, swamp, puddle, drainage channels and plastic containers alongside river banks contributed significantly to the breeding of *An. funestus*. Productivity of puddles for *An. funestus* overlaps between the late wet season and the middle of dry season.

An. moucheti was found to breed in clay pot, canoe, plastic drum / containers, bank of rivers and swamps. Nevertheless, *An. moucheti* were present and more abundant in some breeding sites and not in others. River bank was generally the most productive habitat for *An. moucheti*. They prefer the banks of river during the dry season and swamps during the wet season.

An. nili was found to breed in clay pots, canoe, drainage channels, bank of rivers, plastic tank drum / container and swamps. Nevertheless, *An. nili* were present and more abundant in some breeding sites and not in others. Riverbank was generally the most productive habitat for *An. nili*. In the dry season, riverbank and canoe were the most productive habitat for *An. nili.* In the wet season, drainage channel alongside other breeding habitats were the most productive habitat for *An. nili.*

High productivity or abundance of *Anopheles* mosquito larvae in the river banks may be attributed to its size in addition to its stability. Minakawa *et al* (2005) found out that habitat stability and abundance of *An. gambiae* s. l. was positively correlated with habitat size.This is because smaller habitats normally have low abundance of mosquito larvae and are easily lost than bigger habitats. Frequent use and scooping of water out of canoe also could have contributed to its lower productivity as a breeding site. Otherwise, canoe parked for a long time by travellers can contribute largely to the breeding of *Anopheles* species. Even though other man made containers are used regularly to store water, they are being put in use from time to time, thereby reducing the population of mosquito larvae in them.

The *Anopheles* species were collected in all the months. This is because some of the breeding habitats utilized by them were available in a given month of the year. In species comparison, *An. gambiae* s. s. was significantly the most abundant *Anopheles* species larvae collected from the various breeding sites. *An. funestus* larvae was the second most abundant. *An. moucheti* and *An. nili* larvae were the least and they occurred at the same level. This is possibly due to the fact that *An. gambiae* s. s. and *An. funestus* have more number of breeding sites than *An. moucheti* and *Anopheles nili.* Also, *An. gambiae* s. s. larvae was significantly the most abundant *Anopheles* species larvae collected both in the wet and the dry season. *An. funestus, An. moucheti* and *An. nili* larvae recorded the same level of abundance in the dry season. In the wet season, *An. funestus* larvae recorded significantly higher abundance than *An. moucheti* and *An. nili* larvae. This may be due to additional breeding site of *An. funestus* created during the wet season.

There was no significant difference in the seasonal abundance of the total *Anopheles* species larvae in the study area. It implies that they have the same level of occurrence at various seasons

of the year. This contradicts the findings of Lamidi (2009); Oyewole *et al*. (2010) and Donovan *et al*. (2012) who reported that larval counts and density of *Anopheles* mosquitoes are known to be high during rainy seasons and decline during dry seasons. In the present study, habitats lost to the dry season were largely compensated for by bank of rivers, canoe and water storage containers like plastic drums that were available year round. Within species, *An. funestus*, *An. moucheti* and *An. nili* larvae also showed no significant difference in their seasonal abundance. However, *An. gambiae* s. s. larvae were significantly more abundant in the wet season than in the dry season.

Habitat characteristics such as the physical chemical and biological characteristics of the habitats (Edillo *et al.*, 2006; Muturi *et al.*, 2007; Fillinger *et al.*, 2009; Robert *et al.*, 1998 and Minakawa *et al.*, 2005) are important factors in determining habitat productivity. The breeding habitats in the study area showed some biological characteristics such as the presence of invertebrate organisms, vertebrate organisms, vegetation, algae, bacterial and fungal organisms. Mosquito larvae belonging to other Genera constituted the major invertebrate fauna of *Anopheles* species breeding habitats. Vertebrate organisms present include: fish and tadpole. Algae and vegetations (grasses and leaves from trees) were also identified in the breeding sites. Six different bacterial isolates: *Pseudomonas* species*, Bacillius* species*, Serratia* species*, Micrococcus* species *and E. coli* were found in the breeding sites. Fungal isolates: *Candida* species, *Emmonsia* species, *Chrysonilia* species, *Aspergillus* species, *Trichosporium* species and *Scedosporium* species were identified in the breeding sites. Other mosquito larvae alongside *An. gambiae* s. s., *An. funestus*, *An. moucheti* and *An. nili* larvae were found in sympatry in some breeding sites; but there were breeding sites where only one, two or three of the *Anopheles* species were found. This is similar with report of Levine *et al*. (2004) who reported that *An. gambiae* s. s. and *An. arabiensis*, were broadly sympatric even though only one or the other was found in some areas. The mosquito larvae from other genera may be their competitors for resources within the breeding habitats.

Presence of fish, tadpoles in the water bodies may also help to regulate *Anopheles* species abundance. The vertebrate organisms (tadpole and fish) constitute predators that could reduce the survival and abundance of *Anopheles* species larvae. The nutrient value of the breeding site provides favourable conditions for the breeding of bacteria, fungal spores and algae which

constitute the majority of food the mosquito larvae ingest. Bacterial populations isolated from the breeding sites are similar to those recently reported by Chukalo and Abate (2017). Some of the bacteria, algae and fungi provide food nutrients to *Anopheles* species larvae and help their growth and survival. Bacteria are the most important microbial constituents of mosquito larvae food and the mosquito can grow on culture made only of bacteria (Merritt *et al*., 1992).This is in line with Gimnig *et al*. (2002) who found that adequate food such as algae, bacterial composition and nitrogen were important regulators of *An. gambiae* larval growth. He added that in the absence of predators the highest survival of larva has been observed at 66% algal cover. Algae can also protect the larvae from intense heat of the sun. Vegetation and plant materials found in the breeding habitats of *Anopheles* species may help to ensure nutrient availability for the larvae directly or indirectly. Directly, mosquito larvae can feed on them due to their degradation ability as a result of the activities of microorganisms (bacteria and fungi). Indirectly they provide food for the microorganisms (bacteria, fungi and algae) which are inturn fed upon by the *Anopheles* mosquito larvae. The longer the detritus is present in the larval habitat, the more microbial degradation which in turn might allow an increase in nutrient absorption by the larvae (Cummins and Klug, 1979). On the other hand, some of the bacteria and fungi present in the breeding habitats of *Anopheles* mosquitoes may be entomopathogenic (Priyanka *et al*., 2001; Priyanka and Prakash, 2003; Prakash *et al*., 2010; Verma and Prakash, 2010; Soni and Prakash, 2010; Soni and Prakash, 2011). In this case, they can cause a decline in the populations of different *Anopheles* mosquitoes.

Anopheles mosquitoes were found to breed in water with temperature range of 26.0ºC to 30.2ºC, pH range of 6.77 to 7.68, Biochemical oxygen demand of 18.9 to 98.57 mg/l, Total dissolved solute level of 70.6 to 1136 mg/l, total suspended solute level of 114 to 522 mg/l, chemical oxygen demand concentration of 15.97 to 86.95 mg/l, salt concentration of 0.01 to 0.13 mg/l, dissolved oxygen concentration of 1.91 to 7.22 mg/l, sulphur concentration of 17.28 to 51.2 mg/l, iron concentration of 0.35 to 2.23 mg/l, cadmium concentration of 0.01 to 0.34 mg/l, lead concentration of 0.3 to 1.8 mg/l, manganese concentration of 0.09 to 0.45 mg/l and phosphorus concentration of 0.2 to 2.72 mg/l.

The pH range for *Anopheles* species is similar to pH of 7.4 reported for *Aedes* mosquitoes (Adebote *et al*., 2008; Afolabi *et al*., 2010) and 6.8 to 7.2 required for the weakening of the egg shells of mosquitoes for the first instars larval stage to emerge (Okogun, 2005). Adebote *et al*. (2008) suggested that pH less than 5.0 and slightly higher than 7.4 produced a lethal effect on mosquito species.

No physicochemical factor was found to affect *An. nili* larval abundance. *An. gambiae* s. s. and *An. funestus* larvae showed strong positive correlation with chemical oxygen demand of the breeding sites. Specifically for *An. funestus*, there was significant strong negative correlation between its larval abundance and salinity. *An. moucheti* showed significant strong negative correlation between its larval abundance, and dissolved oxygen as well as iron concentrations of their breeding habitats. It shows that different *Anopheles* species respond to change in the physicochemical characteristics of their breeding habitats unequally and this depends on their tolerance range (Chen *et al.,* 2006). It is possible that all the physicochemical parameters identified in the breeding habitats are within the range of tolerance of *An. nili*. Increase in chemical oxygen demand favours *An. gambiae* s. s. and *An. funestus* larvae survival. A decrease in chemical oxygen demand would be unfavourable to them. It shows that *An. gambiae* s. s. and *An. funestus* can survive in habitats with high level of organic materials, though within certain limits. *An. funestus* is sensitive to salinity levels of their breeding habitats. They prefer breeding habitats with very low level of salt concentration. Some of the aerobic organisms present in the breeding habitats of *Anopheles* mosquitoes might be pathogenic to them thereby reducing their population. *An. moucheti* was equally found to be the most affected by the toxic effect of iron as a pollutant. Ndenga *et al*. (2012) also reported that habitats with low anopheline presence had higher levels of iron. In contrast, Edillo *et al*. (2006) observed significant association between the young larvae of *An. gambiae* s. s. and the following physicochemical parameters of their breeding sites: dissolved oxygen (D.O.), nitrate (NO_3), total alkalinity, turbidity, and water surface temperature.

5.3 Biting and resting behaviors of the adult *Anopheles* species to identify their preferred biting time, biting location and resting location.

All the adult *Anopheles* species identified in the study were found in indoor and outdoor locations; with preference for indoor location both in the dry season and in the wet season. *An. gambiae* s. s was the most abundant species collected inside the house as well as outside the house. *An. funestus, An. moucheti* and *An. nili* occured at the same level inside the house; and also outside the house. In either of the locations, the *Anopheles* species encountered in the study may be biting, resting or biting and resting.

Biting mostly occurs in the evening and early morning between 4.00pm and 6.00am. *An. gambiae* s. s. fed for a longer duration than *An. funestus, An. moucheti* and *An. nili.* They fed between 4.00pm – 6.00am. *An. funestus* feed between 5.00pm – 4.00am. The shortest duration of feeding was observed with *An. moucheti.* They fed between 5.00pm – 12.00am. *An. nili* feed between 5.00pm – 2.00am. It appears that the *Anopheles* species in the study area commenced feeding early and almost at the same time. Their biting time covered the period when people are still awake outdoors and when they move indoors to sleep. This shows their exophagic and endophagic attributes.

It was observed from the study that between 10.00pm and 3.00am, different *Anopheles* species have their peak biting time. It started with *An. funestus* that peaked from 10.00pm – 11.00pm, *An. moucheti* that peaked from 11.00pm – 12.00am, *An. nili* that peaked from 12.00am – 1.00am, *An. funestus* with second peak from 2.00am – 3.00am and finally *An. gambiae* s. s. that peaked from 2.00am – 3.00am. The peak biting times coincided with the period when most people are already asleep. Peak biting by *An. gambiae* and *An. funestus* had been reported between 11.00pm and 05.00am, a period when most people are in bed and under nets if they have them (Maxwell *et al*., 1998). *An. funestus* had its first peak earlier which coincides with the fact that some *Anopheles* species have a biting peak well before midnight (Yohannes and Boelee, 2012, Tirados *et al*., 2006, Geissbűhler *et al.,* 2007). Peak biting for *An. funestus, An. nili* and *An. moucheti* occurred in the middle hours of the night whereas that of *An. gambiae* s. s. occurred in the very early hours of the morning near dawn. *An. funestus* and *An. gambiae* s. s. had been reported to have a biting peak well before midnight (Yohannes and Boelee, 2012., Tirados *et al*., 2006,

Geissbűhler *et al.,* 2007), in the middle hours of the night (Fornadel *et al*., 2010., Taye *et al*., 2006) or in the very early hours of the morning near dawn (Braack *et al*., 1994, Githeko *et al*., 1996) depending on their geographical distribution.

Seasonal variability was also observed in the peak biting time of *Anopheles* species. They bite more in the wet season, especially between April to July than in the dry season. The peak monthly biting time for *An. moucheti* was April. *An. gambiae* s. s. and *An. funestus* had peak monthly biting time in June. The peak monthly biting time for *An. nili* was April. This may be attributed to the increase in population density of the *Anopheles* species and this corresponds to the peak of rains (Okullo *et al*., 2017).

From the study, fed, half gravid, gravid and unfed *Anopheles* species were collected. Significantly higher number of fed female *Anopheles* species was collected than those in other gonotrophic states. This was true for *An. gambiae* s. s., *An. funestus, An. moucheti* and *An. nili*. The number that was unfed, half gravid and gravid remained at the same level for all the *Anopheles* species. This shows that adult female *Anopheles* species shows high frequency of blood feeding. The *Anopheles* species in the study area bite both indoors and outdoors. It is an indication that they show ambivalent feeding behavior. They bite both indoors and outdoors because they are normally found where humans are aggregated (Smith *et al*., 2005). Nevertheless, *Anopheles* mosquitoes showed preference for biting indoors, both in the dry and wet seasons.

An. gambiae s. s. and *An. funestus* showed preference for biting indoors both in the dry and wet seasons. This is similar to the report that *Anopheles* species have preferential feeding habits as some tend to favour feeding indoors, such as the African vectors *An. gambiae* s. s. and *An. funestus* s. s. (Coetzee and Fontenille*,* 2004; Costantini *et al.,* 1999). On the contrary, earlier studies by Sinka *et al.* (2010) supported that *An. gambiae* s. l. bites almost as much outdoors as indoors. *An. moucheti* and *An. nili* had no preferred biting location. It simply means that they can bite in any location regardless of the season.

After biting, the *Anopheles* species in the study area rest both indoors and outdoors. Thus they are both endophilic and exophilic. However, they may show preference for resting outdoors during the dry season. Specifically, *An. gambiae* s. s. and *An. funestus* showed preference for resting outdoors during the dry season only. *An. moucheti* and *An. nili* had no preferred resting location either in the dry or wet season. Walls of the rooms and ceilings provide resting surfaces for the *Anopheles* species. Those resting outside may utilize vegetation and other structures outside the house to rest. Depending on the availability of the resting surfaces, their resting location may change. Naturally, *An*. g*ambiae s. s*. and *An. funestus* are endophilic species (Pates and Curtis, 2005). *An. nili* were known to have low indoor density (Oyewole *et al*., 2010). The resting behavior of *An. moucheti* has not been reported. Outdoor resting by *Anopheles* species, especially *An. gambiae* s. s. and *An. funestus* may be attributed to repellency effect of insecticides (sprayed or in LLINs) when used in indoor location. Shorter indoor resting period and increased exophily have been attributed to insecticide pressure (Rozendaal *et al*., 1989; Darriet, 1991; Mnzava *et al*. 1995). Moreover, preference for outdoor resting especially in the dry season will enable the *Anopheles* mosquitoes utilize habitats such as river banks, swamps, parked canoes for oviposition.

5.4 Entomological indices (*Anopheles* species human blood index and sporozoite rate) to ascertain malaria transmission in the study area.

The human blood index of *Anopheles* species collected and observed in the study was 95.0%. It shows that there is a high level of contact between *Anopheles* species and humans. The remaining *Anopheles* mosquitoes might have fed on non-human hosts within the study area. Domestic animals such as goats, cow, sheep, dogs and pigs have also been reported to also constitute blood meal sources for *Anopheles* species (Logue *et al*., 2016; Mwangangi *et al*., 2003; Ijumba *et al*., 1990). For all *Anopheles* species collected, there was no significant difference between their human blood index in the wet season and that in the dry season. Human IgG was detected in 97.4% of *An. gambiae* s. s., 87.0% of *An. funestus*, 75.4% of *Anopheles moucheti* and 83.3% of *Anopheles nili* collected from the study area. This is in line with the findings of Mwangangi *et al*., (2003) where he reported HBI of 98.97% and 99.48% for *An. gambiae* s. l. and *An. funestus* respectively. Even with the availability of cattle and other

domestic animals in the study area, high proportion of *An. gambiae* s. s., *An. funestus*, *An. moucheti* and *An. nili* were observed to have fed on human. This suggests that humans are the preferred host for *Anopheles* species and it collaborated the findings of Mbogo *et al.* (1993). Comparing the HBI among the species, there were significant differences throughout the study period especially during the wet season. The relative efficiency of *An. gambiae* s. s. was highest for human biting throughout the study period and least for *An. moucheti*. HBI of *An. nili* and *An. funestus* showed varied status; it could be as high as that of *An. gambiae* s. s. or as low as that of *An. moucheti*. In the dry season, some persons may sleep outside the houses and this increase the chances of *An. moucheti* and *An. nili* obtaining blood meals from humans as *An. gambiae* s. s. and *An. funestus* shows preference for indoor biting. The monthly human blood index of *Anopheles* mosquitoes remained at the same level throughout the study period. It implies that man biting occur all year round.

In the course of the bite, an infected female *Anopheles* mosquito may transmit malaria parasites to susceptible individuals or a newly emerged adult female may pick up gametocytes of *Plasmodium* species to become infective. The sporozoite rate of *Anopheles* species observed in the study was 2.5%. The low level of sporozoite rate recorded may be due to the length of time sporozoite development takes place, 10-12 days and the time from the first blood meal to the second blood meal averages only three days. Thus, a mosquito may need three gonotrophic cycles before it becomes infective (WHO, 2003). Before this time, there could be temporal fluctuations in mosquito abundance with attendant alteration in age-structure of the mosquito populations. Therefore, emergence of a large cohort of young adult *Anopheles* mosquitoes would instantly reduce their sporozoite rate in a mosquito population. Also during sporozoite development, the the ookinetes are required to cross layers of epithelial cells in *Anopheles* species midgut within a limited time. This is because, epithelial cells invaded by ookinete die immediately and then ejected from midgut wall, which exposes the parasite to danger of being removed from the epithelium with the damaged cells (Kariu *et al.*, 2006; Ham and Baullas-Mury, 2002; Zieler and Dvorak, 2000). In addition, during the midgut invasion, many ookinetes are killed by the *Anopheles* species defence system and the number of malaria parasite is greatly reduced (Blandin *et al.*, 2004).

For all *Anopheles* species collected, there was no significant difference between the sporozoite rate in the wet season and that in the dry season. There was also no significant difference in the sporozoite rate of the *Anopheles* species with respect to months. This is an indication of possible malaria transmission all year round and it is in concordance with the report of Adeleke *et al*. (2008) and Ndenga *et al*. (2011). The reason is because the *Anopheles* species collected in this study bite all year round.

The sporozoite rates were 2.5%, 3.5%, 2.2% and 1.5% for *An. gambiae* s. s., *An. funestus, An. moucheti* and *An. nili* respectively. Comparing the sporozoite rates among the species, there was no significant differences in the overall, dry season and monthly sporozoite rates of *Anopheles* species. However, the sporozoite rate of the *Anopheles* species differed in the wet season with *An. gambiae* s. s. and *An. funestus* recording the highest. Nevertheless, the present study has confirmed that *An. gambiae* s. s., *An. funestus, An. moucheti* and *An. nili* are suitable vectors for malaria parasite transmission in the study area. *An. gambiae* s. s. and *An. funestus* were identified as the principal malaria vectors. *An. moucheti* and *An. nili* were secondary vectors and are important during dry season transmission.

Earlier, low sporozoite rates of 7.6% and 1.4% had been reported in *An. gambiae* s. s. and *Anopheles arabiensis* respectively during the Garki Malaria Control Programme (Molineaux and Gramiccia, 1980). Similarly, Ogola *et al*. (2017) reported sporozoite rates of 10.0% and 11.8%, 0.0%, and 18.8% among blood-fed *An. gambiae* s. s., *An. arabiensis*, *An. funestus* s.s. and *An. coustani* respectively. In Nigeria Oduola *et al*. (2012), working in Oyo, south-western Nigeria, found that *P. falciparum* sporozoite infection rate of *An. gambiae* s. s. varied between 1.9% and 3.1% in the study communities. However, high sporozoite rates of 31.5% and 17.9% were reported for *An. gambiae* s. l. and *An. funestus* respectively in Makurdi Nigeria (Msugh-Ter *et al*., 2014). Low sporozoite rate in the study area may be attributed to reduced gametocyte density in the blood of humans in the study area.

Variation in the sporozoite rate recorded in this study and the reports of other researchers may be due to difference in the study areas; ranging from prevalence and intensity of malaria within a given area to climatic factors. Factors such as temperature, humidity and rainfall may impact the lifecycle of malaria parasites. Higher temperatures accelerate *Plasmodium* species growth within *Anopheles* mosquitoes (CDC, 2012). Temperature between 15^0C - 40^0C and humidity between 55% and 80% are suitable for the completion of the *P. falciparum* and *P. vivax* malaria parasites life cycle (Zhou *et al*., 2004). However in this study, there was no significant correlation between the climatic factors (temperature, rainfall and relative humidity) and sporozoite rates of *Anopheles* species. It may be because of the fact that the study area was a riverine area with a very narrow temperature range of 26.2ºC – 30.3ºC even though the relative humidity of 64% - 88% falls within that reported by Zhou *et al*. (2004). Rainfall as a factor may only be important to cause rise in the *Anopheles* species population so as to increase their probability of being infective.

An. gambiae s. s. recorded an overall EIR of 0.100 bites/person/night. *An. funestus* recorded an overall EIR of 0.021 bites/person/night. *An. moucheti* recorded an overall EIR of 0.002 bites/person/night. *An. nili* recorded an overall EIR of 0.004 bites/person/night. The difference shown in the EIR of different *Anopheles* species strongly showed that *An. gambiae* s. s. is the major malaria vector in Anambra East LGA. The EIR results of this study is similar to 0.28 infective bites/person/night and 0.76 infective bite/person/night for two different locations in Gabon (Elissa *et al*., 2003). However, it differs from 61.79 and 6.91 bites/person/night for Lunyerere and Nyalenda villages in Kenya (Imbahale *et al*., 2012) and 23, 53 and 61 records in another three different areas of Gabon (Sylla *et al*., 2000). Lower EIR of the study area could be attributed to greater level of compliance in the implementation of malaria control strategies in the study area. The impact of control tools on the EIR has earlier been reported (Geissbuhler *et al*., 2009; Gu *et al*., 2006). It equally shows that not every bite from *Anopheles* mosquitoes leads to malaria parasite transmission. There was no seasonal variation in the EIR of the different *Anopheles* species. However, *An. moucheti* and *An. nili* were only important in the dry season transmission.

5.5 Malaria endemicity through monthly and seasonal prevalence / intensity studies of malaria with respect to age, gender, month and seasons of the year.

The study recorded 61.2% malaria parasite prevalence due to *P. falciparum* and with mean intensity of 263.4±3.2 malaria parasites / µl of blood. *P. falciparum* encountered in the study is the most commonly encountered species in Nigeria (Onyido *et al.,* 2011; Mbanugo and Ejims, 2000). Among the communities studied, there is same level of malaria parasite prevalence and intensity. This is similar to the prevalence of 61.7% (Egbuche *et al*., 2013) record of an earlier study within the same area. It suggests stable malaria transmission where the adult population may have shown a high level of immunity (WHO, 2003). Among the communities studied, malaria parasite prevalence occurred throughout the year. This is an indication that people living in the study area may be experiencing at least one episode of malaria per year. It supports the report of WHO (2003) that estimated as much as four bouts per person per year on the average. Both males and females, and people of all ages were equally susceptible to malaria parasite infection. However, the prevalence of malaria parasites was found to depend on months and also on seasons of the year. The wet season recorded significantly higher prevalence of malaria parasite than the dry season. The month of April recorded significantly the highest prevalence of malaria parasites than other months. Within the dry season, prevalence of malaria parasite was highest in January. Opposed to the higher prevalence of malaria parasite in the wet season, intensity of malaria parasite infection was significantly higher in the dry season. Higher prevalence during the wet season may be attributed to stability of *Anopheles* species breeding sites during the wet season. During the period, *Anopheles* species population tends to be high with increase chances of malaria parasite transmission. On the hand, higher intensity of malaria parasite infection during the dry season may be attributed to ignorance and low level of compliance to malaria control tools during the dry season. Ordinarily, most people especially the rural communities believe that malaria parasite transmission occurs only during the rainy season. So during that period, they may take some prophylactic and chemotherapeutic drugs to prevent occurrence of malaria thereby reducing the wet season intensity of malaria parasite infection. Some would have parts of their body exposed to the bite of *Anopheles* species due to hot weather during the dry season. In a case where there is successful transmission of malaria parasite to an individual by more than one infected *Anopheles* species, malaria parasite intensity will be higher.

Both males and females suffer from malaria parasite infection at the same level in all the seasons and throughout the year. In the same vein, all age groups suffer from malaria parasite infection at the same level in all the seasons and throughout the year. Malaria parasite intensity increases at the point between end of wet season (October) and onset of dry season (November). It reduces gradually from the dry season through the wet season. In the wet season alone, the intensity of malaria parasite infection is significantly highest in October, lower in April and least in May, June, July, August and September. Thus severity of malaria parasite infection occurs at the early and the late periods of wet season. In the dry season alone, the intensity of malaria parasite infection is significantly higher in November, and least in February and March. The intensity level decreases as the dry season progresses with fluctuating levels in January and December.

High parasite rate of 61.2%, mean intensity of 263.4±3.2 malaria parasites / µl of blood, all year round transmission of malaria parasite categorizes the study area as a hyperendemic area. The communities within the study area presents with the same level of endemicity because they have similar characteristics of malaria distribution. This could be attributed to the fact that the communities have the same climatic, environmental and ecological features. This is because, the differences in malaria risk among the sites can be explained by topography, terrain characteristic (Atieli *et al*., 2011), and environmental conditions among others. Prevalence of malaria parasite infection in the study area confirms that human hosts carrying *P. falciparum* are found in the area. In addition, infected *Anopheles* species are present there. It shows that they are part of the major factors that affect malaria prevalence, intensity and endemicty in the study area. Ani (2004) had earlier stated that Malaria infection is mostly acquired in areas where human hosts carrying the *Plasmodium* parasites are found in addition to presence of enough Anopheline mosquitoes under suitable environmental conditions.

The differences in malaria risk among the sites can be explained by vector species of local importance and availability of breeding habitats (Imbahale *et al*., 2011). Breeding of mosquito could be traced to poor sanitation which may contribute to high prevalence and intensity of malaria. A strong positive correlation has been observed between malaria prevalence and abundance of *Anopheles* species in Ogbunike, Anambra state (Onyido *et al*, 2011). Malaria in the

study area is transmitted by a range of *Anopheles* mosquitoes that comprises: *An. gambiae* s. s., *An. funestus, An. moucheti and Anopheles nili*. These constitutes *Anopheles* species of local importance in Anambra East LGA of Anambra State. *An. gambiae* s. l. Giles (Diptera: Culicidae) is one of the most important malaria vectors in Africa, where 90% of the world malaria cases occur (Chandre *et al*., 1999). The anthropophilic, endophilic, exophilic, endophagic and exophagic nature of these *Anopheles* species observed in the study may contribute to their efficiency in malaria parasite transmission.

In this study, Environmental temperature and relative humidity had no significant effect in the prevalence and intensity of malaria parasite infection in the study area. The temperature range of 26.2ºC – 30.3ºC in the study area was optimum for the survival of *Anopheles* species because the highest proportion of malaria vectors surviving the incubation period is observed at temperatures between 28°C - 32°C (Craig *et al*., 1999; Jonathan *et al*., 2006). Rainfall had significant strong negative correlation with malaria parasite intensity. Increase in the amount of rainfall tends to reduce the intensity of malaria parasite infection even though the prevalence level seems the same. The period is characterized by reduced environmental temperature that may increase the developmental time of malaria parasites in both humans and the vectors. It also corresponds with the period when, people take some prophylactic and chemotherapeutic drugs to prevent occurrence of malaria.

There was no significant correlation between malaria parasite prevalence and the sporozoite rates of the different *Anopheles* species. It points to the probability of malaria parasite transmission where one or few infected *Anopheles* species are as dangerous as many infected individuals of the same population. The probability of infection increases with the number of infectious bites. Uneven distribution of mosquito bites between hosts could also be a factor (Churcher *et al*., 2014). Fewer number of infected *Anopheles* species biting a long chain of susceptible humans may lead to higher prevalence than large population of infected *Anopheles* species biting already infected individuals. There was also no significant correlation between malaria parasite intensity and sporozoite rates of different *Anopheles* species. It may be that the infective *Anopheles* species in the study area harbored the same number of parasites. Even if the number is not the same, it may be that *Anopheles* species with salivary gland sporozoites are equally infectious

irrespective of the number of parasites they harbor. However, mosquitoes with a higher number of sporozoites in their salivary glands following blood-feeding were reported to have caused infection and would have done so quicker than mosquitoes with fewer parasites (Churcher *et al*., 2017). Reversibly, Gametocyte density in the host blood is a determinant of the infection success in the mosquito. More efficient transmission represents more mosquitoes being infected. Thus in measuring infection rates of *Anopheles* species, it is possible that the probability of malaria parasite being transmitted from a single infectious human to a mosquito and the proportion of mosquitoes in a population that become infected after biting a human is irregular.

CONCLUSION

The study has revealed that *An. gambiae* s. s., *An. funestus, An. moucheti* and *An. nili* are the malaria vectors present in Anambra East Local Government of Anambra State. These *Anopheles* species were found all through the year with varied peaks attributed to the difference in the amount of rainfall during the seasons. They utilize both natural and man-made habitats for breeding. The habitats included: basin, broken clay pots, plastic drums / containers, canoes, drainage channels, river banks, head pans, plastic drums, plastic tank cover, puddles and swamps. Most of the breeding habitats identified were available all year round and were utilized by all the *Anopheles* species. Each *Anopheles* species encountered in the study area used multiple breeding sites for oviposition but may show distinct preference for certain type of breeding habitat over the other. *An. gambiae* s. s. and *An. funestus* utilize exactly the same breeding habitats whereas *An. moucheti and An. nili* utilize exactly the same breeding habitats. Nevertheless, River bank was generally the most productive habitat for *An. gambiae* s. s., *An. funestus*, *An. moucheti* and *An. nili*. The breeding habitats showed some biological characteristics such as the presence of invertebrate organisms, vertebrate organisms, vegetation, algae, bacterial and fungal organisms, which could affect the larval abundance. *Anopheles* species were found to breed in water with suitable temperature, pH, Biochemical oxygen demand, Total dissolved solute level, total suspended solute level, chemical oxygen demand concentration, salt concentration, dissolved oxygen concentration, sulphur concentration, iron concentration, cadmium concentration, lead concentration, manganese concentration and phosphorus concentration. However, No physicochemical factor was found to affect *An. nili* larval abundance. *An. gambiae* s. s. and *An. funestus* larvae showed strong positive correlation with chemical oxygen demand of the breeding sites. Specifically for *An. funestus*, there was significant strong negative correlation between its larval abundance and salinity. *An. moucheti* showed significant strong negative correlation between its larval abundance, as well as iron concentration of their breeding habitats.

All adult of the *Anopheles* species identified in the study were found in indoor and outdoor locations throughout the study period. It is an indication that these *Anopheles* species show ambivalent feeding and resting behavior. It appears that the *Anopheles* species in the study area

commence feeding early and almost at the same time. Their biting time covers the period when people are still awake outdoors and when they move indoors to sleep. This confirms their exophagic and endophagic attributes. The human blood index of *Anopheles* species collected and observed in the study was 95.0%. It shows that there is a high level of contact between *Anopheles* species and humans. This suggests that humans are the preferred host for *Anopheles* species. The sporozoite rate of *Anopheles* species observed in the study was 2.5%. For all *Anopheles* species collected, there was no significant difference between the sporozoite rate among them in the wet season and that in the dry season. This is an indication of possible malaria transmission all year round. *An. gambiae* s. s. and *An. funestus* were identified as the principal malaria vectors in the study area because of their high sporozoite rate in both the dry and the wet seasons. *An. moucheti* and *An. nili* were secondary vectors and were important during dry season transmission.

The study recorded 61.2% malaria parasite prevalence due to *P. falciparum* and with mean intensity of 263.4±3.2 malaria parasites / µl of blood. High parasite rate, high mean intensity and all year round transmission with significantly higher prevalence of malaria parasite during the rainy season categorizes the study area as a hyperendemic area. Thus it suggests stable malaria transmission in Anambra East LGA of Anambra State. The major factors that affect malaria prevalence, intensity and endemicty in the study area include: presence of human hosts carrying *P. falciparum*, presence of infected *Anopheles* species, increased amount of rainfall, multiplicity of *Anopheles* species breeding sites, and anthropophilic, endophilic, exophilic, endophagic and exophagic nature of these *Anopheles* species that contribute to their efficiency in malaria parasite transmission.

RECOMMENDATION.

The study recommends community enlightenment programme on all year round transmission of malaria parasite and the need for adequate use of protective measures in both indoor and outdoor locations. Further studies from this research are also recommended. They include:

1. Determination of nutritional and mosquiticidal effects of the fungal and bacteria isolates of the breeding sites on the survival of *Anopheles* speies larvae.
2. Assessment of parity rates of unfed populations of *Anopheles* species inorder to determine their lifespan and significance in malaria parasite transmission.
3. Identification of blood meal hosts of *Anopheles* species other than humans in the study area.
4. Relationship between parity of *Anopheles* species and their infectivity status.
5. Quantification of sporozoite rate of *Anopheles* species to know the probable amount of sporozoite inoculated on an individual in the course of a blood meal.
6. Detremination of gametocyte density as a measure of *Anopheles* mosquito sporozoite rate.
7. Immunological status of individuals living in different malaria endemic areas.

CONTRIBUTIONS TO KNOWLEDGE

The study has provided a baseline data on the ecology of *Anopheles* mosquitoes and malaria endemicity in Anambra East Local Government Area. It puts the following to limelight:

1. *An. gambiae* s. s., *An. funestus, An. moucheti* and *An. nili* are local populations of *Anopheles* species found in the study area.

2. Each *Anopheles* species has multiple breeding habitats which are of different sizes; and also include both natural and artificial water collections.

3. Different *Anopheles* species respond differently to change in the physicochemical characteristics of their breeding habitats depending on their tolerance range.

4. Vegetations, invertebrate organisms, vertebrate organisms, bacteria and fungi are found in the breeding habitats of the *Anopheles* mosquitoes.

5. Adult *Anopheles* mosquitoes bite all year round; in the evening and early morning between 4.00pm and 6.00am, with peak between 10.00pm and 3.00am.

6. *An. gambiae* s. s. and *An. funestus* showed preference for biting indoors both in the dry and wet seasons.

7. *An. moucheti* and *An. nili* showed no preference for indoor or outdoor biting.

8. *An. gambiae* s. s. and *An. funestus* showed preference for resting outdoors in the dry season only.

9. *An. moucheti and An. nili* showed no preference for resting indoor during the wet season, but may rest in any location during the dry season.

10. Humans are the preferred host of *Anopheles s*pecies in the study area.

11. Infective *Anopheles* mosquitoes were found all year round even though the sporozoite rate is low.

12. Low EIR in the study was an indication that not every bite leads transmission of malaria parasite.

13. People living in the study area may experience at least one episode of malaria in a year; with marked seasonal and monthly variations.

14. The study area is hyperendemic for malaria.

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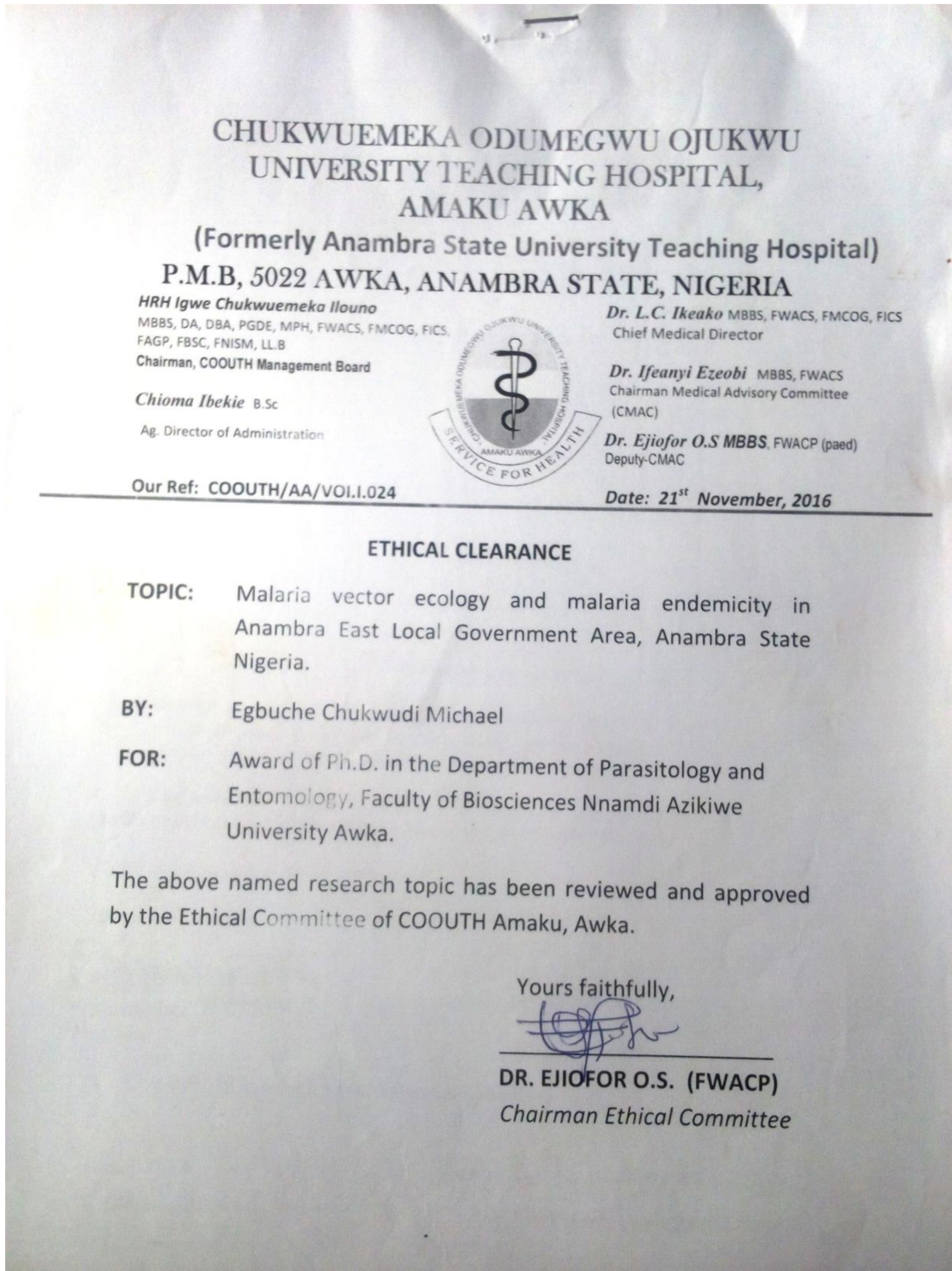
Appendix 1: Ethical Approval letter



CHUKWUEMEKA ODUMEGWU OJUKWU
UNIVERSITY TEACHING HOSPITAL,
AMAKU AWKA
(Formerly Anambra State University Teaching Hospital)
P.M.B, 5022 AWKA, ANAMBRA STATE, NIGERIA

HRH Igwe Chukwuemeka Ilouno
MBBS, DA, DBA, PGDE, MPH, FWACS, FMCOG, FICS, FAGP, FBSC, FNISM, LL.B
Chairman, COOUTH Management Board

Chioma Ibekie B.Sc
Ag. Director of Administration

Dr. L.C. Ikeako MBBS, FWACS, FMCOG, FICS
Chief Medical Director

Dr. Ifeanyi Ezeobi MBBS, FWACS
Chairman Medical Advisory Committee (CMAC)

Dr. Ejiofor O.S MBBS, FWACP (paed)
Deputy-CMAC

SERVICE FOR HEALTH

Our Ref: COOUTH/AA/VOl.I.024

Date: 21st November, 2016

ETHICAL CLEARANCE

TOPIC: Malaria vector ecology and malaria endemicity in Anambra East Local Government Area, Anambra State Nigeria.

BY: Egbuche Chukwudi Michael

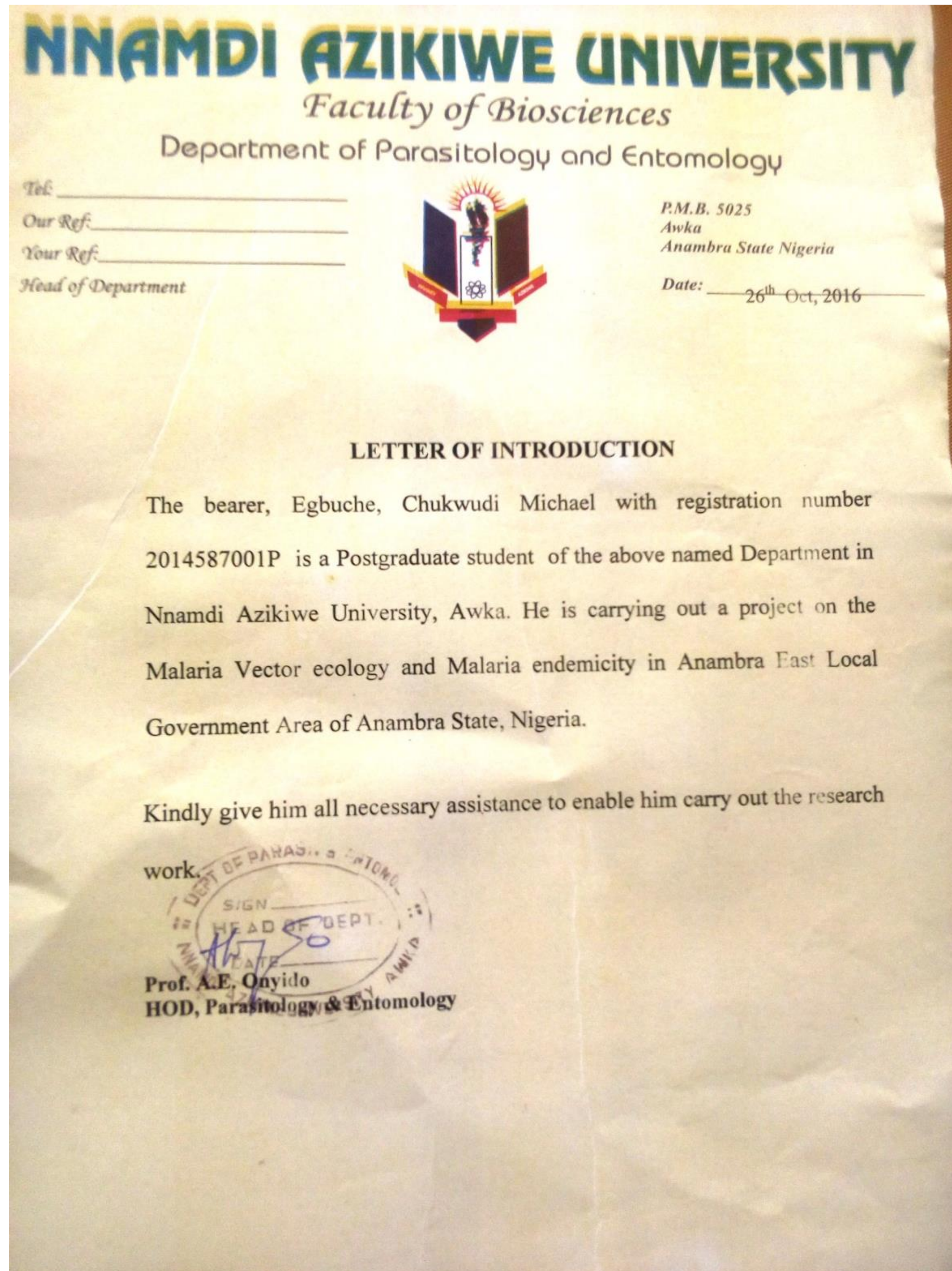
FOR: Award of Ph.D. in the Department of Parasitology and Entomology, Faculty of Biosciences Nnamdi Azikiwe University Awka.

The above named research topic has been reviewed and approved by the Ethical Committee of COOUTH Amaku, Awka.

Yours faithfully,

DR. EJIOFOR O.S. (FWACP)
Chairman Ethical Committee

Appendix 2: Letter of Introduction



NNAMDI AZIKIWE UNIVERSITY
Faculty of Biosciences
Department of Parasitology and Entomology

Tel: ______
Our Ref: ______
Your Ref: ______
Head of Department

P.M.B. 5025
Awka
Anambra State Nigeria

Date: 26th Oct, 2016

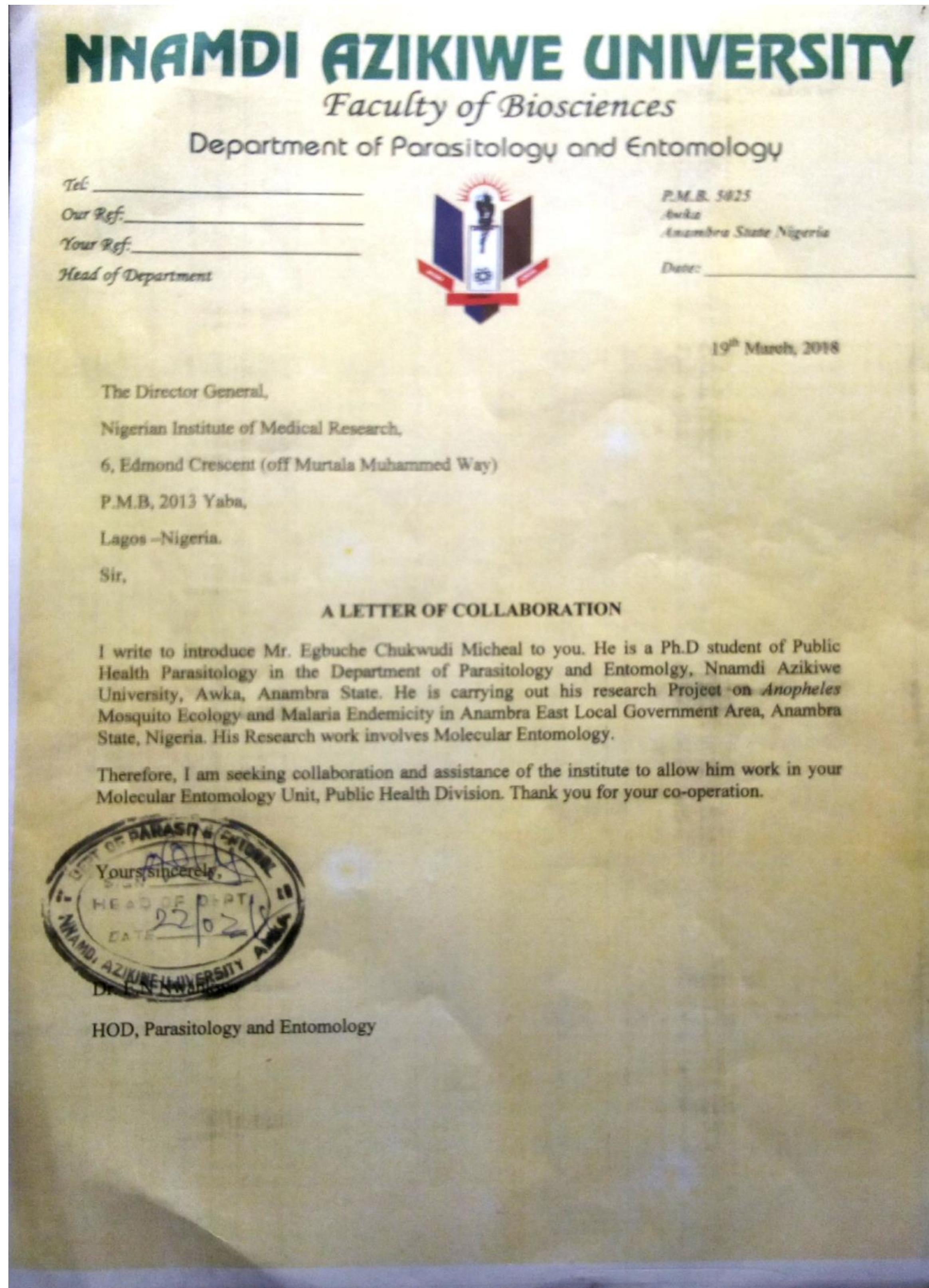
LETTER OF INTRODUCTION

The bearer, Egbuche, Chukwudi Michael with registration number 2014587001P is a Postgraduate student of the above named Department in Nnamdi Azikiwe University, Awka. He is carrying out a project on the Malaria Vector ecology and Malaria endemicity in Anambra East Local Government Area of Anambra State, Nigeria.

Kindly give him all necessary assistance to enable him carry out the research work.

Prof. A.E. Onyido
HOD, Parasitology & Entomology

Appendix 3a: Letter of Collaboration



NNAMDI AZIKIWE UNIVERSITY

Faculty of Biosciences

Department of Parasitology and Entomology

Tel: ____________

Our Ref: ____________

Your Ref: ____________

Head of Department

P.M.B. 5025
Awka
Anambra State Nigeria

Date: ____________

19th March, 2018

The Director General,

Nigerian Institute of Medical Research,

6, Edmond Crescent (off Murtala Muhammed Way)

P.M.B, 2013 Yaba,

Lagos –Nigeria.

Sir,

A LETTER OF COLLABORATION

I write to introduce Mr. Egbuche Chukwudi Micheal to you. He is a Ph.D student of Public Health Parasitology in the Department of Parasitology and Entomolgy, Nnamdi Azikiwe University, Awka, Anambra State. He is carrying out his research Project on *Anopheles* Mosquito Ecology and Malaria Endemicity in Anambra East Local Government Area, Anambra State, Nigeria. His Research work involves Molecular Entomology.

Therefore, I am seeking collaboration and assistance of the institute to allow him work in your Molecular Entomology Unit, Public Health Division. Thank you for your co-operation.

Yours sincerely,

DEPT OF PARASITOLOGY & ENTOMOLOGY, NNAMDI AZIKIWE UNIVERSITY AWKA
SIGN
HEAD OF DEPT
DATE 22/02/

Dr. E.N Nwankwo

HOD, Parasitology and Entomology

Appendix 3b: Approval letter for collaboration

NIGERIAN INSTITUTE OF MEDICAL RESEARCH

FEDERAL MINISTRY OF HEALTH

Prof. Babatunde L. Salako
MBBS (Ib), FWACP, FRCP (Edin), FRCP (Lond), MNIM
Director-General/CEO

6, Edmund Crescent,
(off Murtala Mohammed Way)
P.M.B. 2013, Yaba, Lagos,
Lagos - Nigeria.
Tel: +234-9092133886
E-mail: info@nimr.gov.ng
Website: www.nimr.gov.ng

Our Ref: MR/GEN.AD/1032/V/1341

Date: 17th April, 2018

Dr. E. N. Nwankwo
HOD, Parasitology and Entomology
Faculty of Biosciences
Department of Parasitology and Entomology,
Nnamdi Azikiwe University,
P.M.B. 5025, Awka,
Anambra State Nigeria.

RE: APPLICATION FOR BENCH SPACE IN THE DEPARTMENT OF PUBLIC HEALTH AND EPIDEMIOLOGY TO CARRY OUT Ph.D. RESEARCH PROJECT

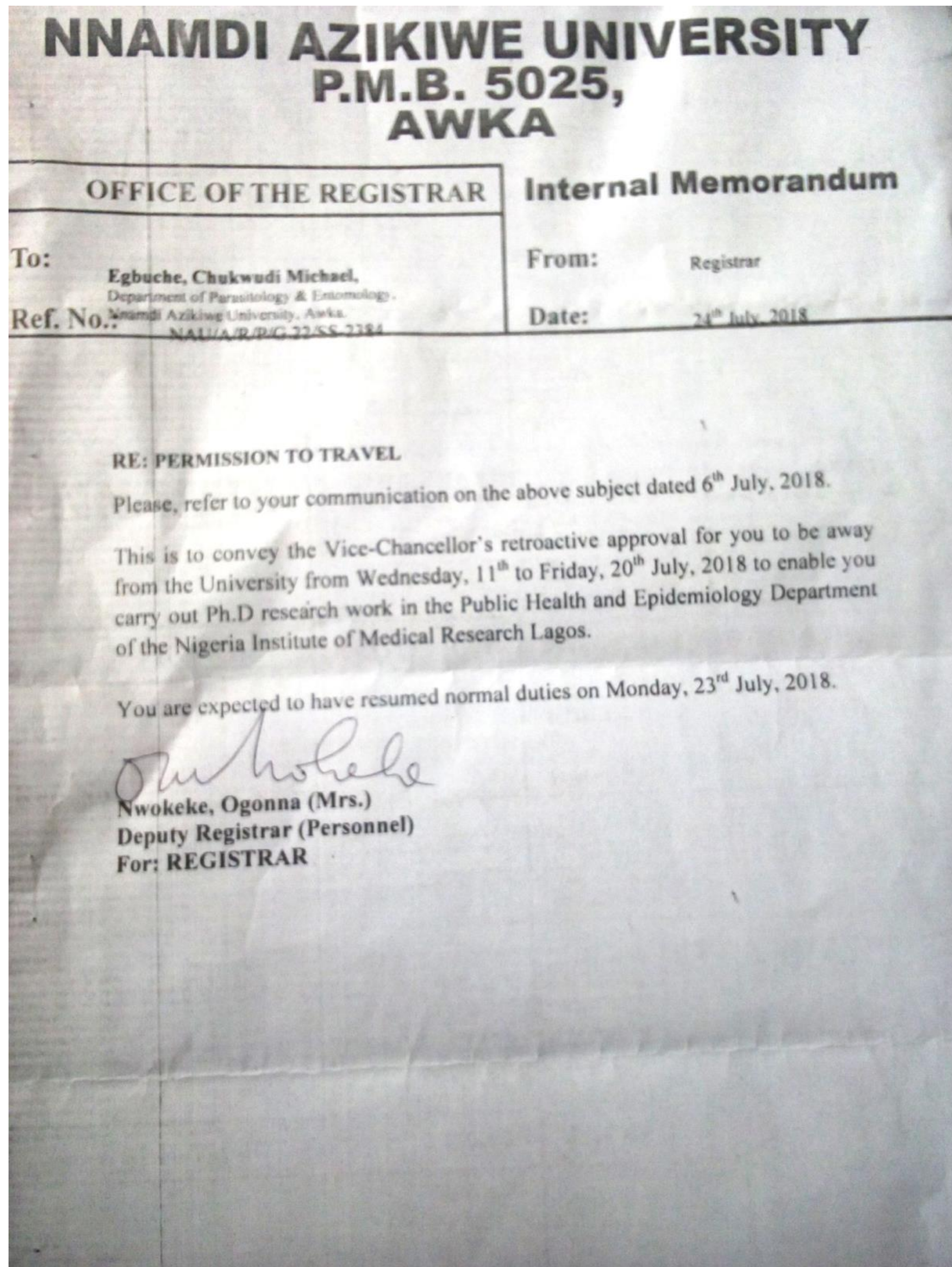
I am directed to refer to your letter dated 19th March, 2018 on the above subject matter and to convey the Director-General's approval of your request to enable Mr. Egbuche Chukwudi Michael carry out Ph.D. Research work in the Public Health and Epidemiology Department of the Institute.

However, your candidate is expected to pay the Bench space fee of **₦250,000.00 (Two Hundred and Fifty thousand naira only)** to the Finance and Accounts Department of the Institute before the commencement of the project.

Thank you.

B. N. Osuji
CEO (GAT&D)
for: Director-General

Appendix 3c: Letter of permission to travel



NNAMDI AZIKIWE UNIVERSITY
P.M.B. 5025,
AWKA

OFFICE OF THE REGISTRAR | **Internal Memorandum**

To: **Egbuche, Chukwudi Michael,**
Department of Parasitology & Entomology,
Nnamdi Azikiwe University, Awka.

From: Registrar

Ref. No.: NAU/A/R/P/G.22/SS-2384

Date: 24th July, 2018

RE: PERMISSION TO TRAVEL

Please, refer to your communication on the above subject dated 6th July, 2018.

This is to convey the Vice-Chancellor's retroactive approval for you to be away from the University from Wednesday, 11th to Friday, 20th July, 2018 to enable you carry out Ph.D research work in the Public Health and Epidemiology Department of the Nigeria Institute of Medical Research Lagos.

You are expected to have resumed normal duties on Monday, 23rd July, 2018.

Nwokeke, Ogonna (Mrs.)
Deputy Registrar (Personnel)
For: REGISTRAR

Appendix 4: Climatic data from NIMET

NIGERIAN METEOROLOGICAL AGENCY

Pope John Paul Street Off Gana Street, Maitama District, Abuja-Nigeria.
Tel/Fax: 234-9-8100339, Fax: 234-1-2636097
Tel: 234-9-5238357, 234-1-2633371,
Tel: 234-9-8100405

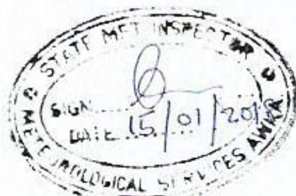
15th January, 2018.

Mr C. M. Egbuche
Department of Parasitology and Entomology
Faculty of Biosciences
Nnamdi Azikiwe University, Awka.

TEMPERATURE, RAINFALL AND RELATIVE HUMIDITY INFORMATION OF ANAMBRA STATE IN THE YEAR, 2016 AND 2017

Month	Temperature		Rainfall		Relative Humidity	
	2016	2017	2016	2017	2016	2017
January	28.4	27.4	0.0	3.4	51	69
February	29.8	30.3	26.0	0.0	65	64
March	29.9	29.2	122.6	33.4	65	73
April	28.4	29.2	187.0	519.9	81	80
May	27.8	28.2	229.2	299.5	79	83
June	27.1	26.9	282.2	680.8	83	85
July	26.2	26.2	239.6	651.8	88	86
August	25.7	26.2	419.0	407.4	89	88
September	25.9	26.6	221.6	598.2	87	88
October	26.7	27.3	264.8	331.6	84	85
November	27.8	28.8	42.7	90.1	80	79
December	27.8	28.0	0.0	0.0	72	68

KEY NOTES: Monthly mean of temperature is reported in degree Celsius (^{0}C). Monthly mean of rainfall is reported in millimeters (mm). Monthly mean of relative humidity is reported in percent (%).



Appendix 5: ANOVA comparing the abundance of *Anopheles* mosquito adults and larvae collected.

```
ONEWAY Abundance BY Anopheles mosquitoes
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Total Population of *Anopheles* collected

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Larvae	3	1375.6667	52.44362	30.27834	1245.3895	1505.9438	1341.00	1436.00
Adult	3	1351.3333	290.82010	167.90507	628.8961	2073.7705	1112.00	1675.00
Total	6	1363.5000	187.37209	76.49434	1166.8651	1560.1349	1112.00	1675.00

ANOVA

Total Population of *Anopheles* collected

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	888.167	1	888.167	.020	.893
Within Groups	174653.333	4	43663.333		
Total	175541.500	5			

Appendix 6: ANOVA comparing the abundance of different *Anopheles* species collected.

```
ONEWAY Abundance BY Anopheles species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Abundance of Anopheles species in the study area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Anopheles gambiae s. s.	12	477.8333	134.89850	38.94184	392.1229	563.5438	267.00	667.00
Anopheles funestus	12	124.5833	44.51038	12.84904	96.3028	152.8639	64.00	180.00
Anopheles moucheti	12	43.5833	13.65456	3.94173	34.9076	52.2590	24.00	65.00
Anopheles nili	12	36.7500	15.24422	4.40063	27.0643	46.4357	19.00	60.00
Total	48	170.6875	195.33279	28.19386	113.9688	227.4062	19.00	667.00

ANOVA

Abundance of *Anopheles* species in the study area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1566706.562	3	522235.521	101.417	.000
Within Groups	226573.750	44	5149.403		
Total	1793280.312	47			

Post Hoc Tests

Abundance of *Anopheles* species in the study area

Student-Newman-Keuls[a]

Different Anopheles species collected in the study area	N	Subset for alpha = 0.05: 1	2	3
Anopheles nili	12	36.7500		
Anopheles moucheti	12	43.5833		
Anopheles funestus	12		124.5833	
Anopheles gambiae s. s.	12			477.8333
Sig.		.817	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 7: Monthly abundance of *Anopheles* mosquitoes in the study area.

```
ONEWAY Population BY Month
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles* mosquitoes

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Nov	12	16.8333	21.47232	6.19853	3.1905	30.4762	1.00	64.00
Dec	12	19.8333	26.04483	7.51850	3.2852	36.3814	1.00	80.00
Jan	12	25.3333	33.53244	9.67998	4.0278	46.6388	3.00	92.00
Feb	12	26.0000	38.88444	11.22497	1.2940	50.7060	1.00	113.00
Mar	12	30.6667	46.15455	13.32367	1.3415	59.9919	.00	138.00
Apr	12	32.1667	47.50470	13.71343	1.9836	62.3497	3.00	161.00
May	12	44.9167	63.97366	18.46760	4.2697	85.5636	4.00	189.00
Jun	12	31.0833	51.88179	14.97698	-1.8808	64.0474	2.00	184.00
Jul	12	28.6667	39.46076	11.39134	3.5945	53.7388	1.00	128.00
Aug	12	26.5833	37.15926	10.72695	2.9735	50.1932	1.00	107.00
Sept	12	22.7500	30.04580	8.67347	3.6598	41.8402	1.00	83.00
Oct	12	34.0000	48.67144	14.05023	3.0756	64.9244	1.00	125.00
Total	144	28.2361	40.94854	3.41238	21.4909	34.9813	.00	189.00

ANOVA

Abundance of *Anopheles* mosquitoes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7055.972	11	641.452	.364	.968
Within Groups	232724.000	132	1763.061		
Total	239779.972	143			

Appendix 8: ANOVA comparing *Anopheles* mosquito abundance between the dry and the wet seasons.

```
ONEWAY Anopheles mosquito BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles* species

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	541.6000	191.88095	85.81177	303.3483	779.8517	387.00	859.00
Wet season	7	781.8571	117.27664	44.32640	673.3943	890.3199	588.00	934.00
Total	12	681.7500	190.25252	54.92117	560.8693	802.6307	387.00	934.00

ANOVA

Abundance of *Anopheles* species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	168360.193	1	168360.193	7.327	.022
Within Groups	229796.057	10	22979.606		
Total	398156.250	11			

Appendix 9: Monthly abundance of different *Anopheles* species collected from the study area

```
ONEWAY Population BY Anopheles species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Abundance of *Anopheles* mosquitoes

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
An. gambiae s. s.	36	90.3333	38.21668	6.36945	77.4027	103.2640	35.00	189.00
An. funestus	36	13.6667	5.30229	.88372	11.8726	15.4607	6.00	32.00
An. moucheti	36	3.9444	1.98486	.33081	3.2729	4.6160	1.00	9.00
An. nili	36	5.0000	3.38062	.56344	3.8562	6.1438	.00	13.00
Total	144	28.2361	40.94854	3.41238	21.4909	34.9813	.00	189.00

ANOVA

Abundance of *Anopheles* species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	187140.083	3	62380.028	165.905	.000
Within Groups	52639.889	140	375.999		
Total	239779.972	143			

Post Hoc Tests

Abundance of *Anopheles* species

Student-Newman-Keuls[a]

Different species of *Anopheles* mosquitoes collected	N	Subset for alpha = 0.05	
		1	2
An. moucheti	36	3.9444	
An. nili	36	5.0000	
An. funestus	36	13.6667	
An. gambiae s. s.	36		90.3333
Sig.		.088	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 36.000.

Appendix 10: Monthly abundance of *Anopheles gambiae* s. s. collected from the study area

```
ONEWAY Anopheles gambiae s. s. Population BY Month
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles gambiae* s. s.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Nov	3	51.0000	11.53256	6.65833	22.3515	79.6485	42.00	64.00
Dec	3	59.3333	22.72297	13.11911	2.8863	115.7803	35.00	80.00
Jan	3	79.3333	16.25833	9.38675	38.9454	119.7213	61.00	92.00
Feb	3	85.3333	34.99047	20.20176	-1.5878	172.2545	46.00	113.00
Mar	3	102.6667	35.50117	20.49661	14.4769	190.8565	67.00	138.00
Apr	3	101.3333	51.69462	29.84590	-27.0832	229.7499	70.00	161.00
May	3	147.3333	36.50114	21.07394	56.6595	238.0072	121.00	189.00
Jun	3	97.6667	74.76853	43.16763	-88.0687	283.4020	54.00	184.00
Jul	3	88.6667	34.07834	19.67514	4.0114	173.3220	68.00	128.00
Aug	3	86.3333	18.00926	10.39765	41.5959	131.0708	74.00	107.00
Sept	3	71.3333	10.40833	6.00925	45.4776	97.1891	63.00	83.00
Oct	3	113.6667	15.50269	8.95048	75.1559	152.1775	96.00	125.00
Total	36	90.3333	38.21668	6.36945	77.4027	103.2640	35.00	189.00

ANOVA

Abundance of *Anopheles gambiae* s. s.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21462.667	11	1951.152	1.579	.169
Within Groups	29655.333	24	1235.639		
Total	51118.000	35			

Appendix 11: ANOVA comparing *Anopheles gambiae* s. s abundance between the dry and the wet seasons.

```
NEW FILE.
DATASET NAME DataSet1 WINDOW=FRONT.
DATASET ACTIVATE DataSet0.
DATASET CLOSE DataSet1.
ONEWAY Agambiae BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles gambiae* s.s

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	366.6000	123.61553	55.28255	213.1110	520.0890	267.00	571.00
Wet season	7	557.2857	74.15910	28.02950	488.7000	625.8714	469.00	667.00
Total	12	477.8333	134.89850	38.94184	392.1229	563.5438	267.00	667.00

ANOVA

Abundance of *Anopheles gambiae* s.s

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106053.038	1	106053.038	11.268	.007
Within Groups	94120.629	10	9412.063		
Total	200173.667	11			

Appendix 12: Monthly abundance of *Anopheles funestus* collected from the study area

```
ONEWAY Anopheles funestus Population BY Month
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles funestus*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Nov	3	10.3333	2.51661	1.45297	4.0817	16.5849	8.00	13.00
Dec	3	11.6667	4.72582	2.72845	-.0729	23.4062	8.00	17.00
Jan	3	13.0000	3.00000	1.73205	5.5476	20.4524	10.00	16.00
Feb	3	9.3333	2.51661	1.45297	3.0817	15.5849	7.00	12.00
Mar	3	12.3333	1.52753	.88192	8.5388	16.1279	11.00	14.00
Apr	3	15.6667	6.42910	3.71184	-.3041	31.6374	11.00	23.00
May	3	19.6667	1.52753	.88192	15.8721	23.4612	18.00	21.00
Jun	3	16.6667	13.61372	7.85988	-17.1517	50.4850	6.00	32.00
Jul	3	15.6667	6.65833	3.84419	-.8735	32.2069	10.00	23.00
Aug	3	12.6667	5.50757	3.17980	-1.0149	26.3482	9.00	19.00
Sept	3	14.0000	1.73205	1.00000	9.6973	18.3027	13.00	16.00
Oct	3	13.0000	1.73205	1.00000	8.6973	17.3027	12.00	15.00
Total	36	13.6667	5.30229	.88372	11.8726	15.4607	6.00	32.00

ANOVA

Abundance of *Anopheles funestus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	272.000	11	24.727	.834	.610
Within Groups	712.000	24	29.667		
Total	984.000	35			

Appendix 13: ANOVA comparing *Anopheles funestus* abundance between the dry and the wet seasons.

```
ONEWAY An. funestus BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles funestus*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	100.8000	45.77882	20.47291	43.9581	157.6419	65.00	180.00
Wet season	7	141.2857	37.45982	14.15848	106.6412	175.9303	64.00	176.00
Total	12	124.4167	44.29541	12.78698	96.2727	152.5606	64.00	180.00

ANOVA

Abundance of *Anopheles funestus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4780.688	1	4780.688	2.845	.123
Within Groups	16802.229	10	1680.223		
Total	21582.917	11			

Appendix 14: Monthly abundance of *Anopheles moucheti* collected from the study area

```
GET
  FILE='C:\Users\PC\Desktop\anopheles population by month town and
species.sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
ONEWAY Population BY Month
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles moucheti*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Nov	3	3.0000	1.00000	.57735	.5159	5.4841	2.00	4.00
Dec	3	5.0000	1.73205	1.00000	.6973	9.3027	4.00	7.00
Jan	3	3.3333	.57735	.33333	1.8991	4.7676	3.00	4.00
Feb	3	3.6667	1.52753	.88192	-.1279	7.4612	2.00	5.00
Mar	3	5.0000	1.73205	1.00000	.6973	9.3027	3.00	6.00
Apr	3	5.0000	1.00000	.57735	2.5159	7.4841	4.00	6.00
May	3	5.6667	2.08167	1.20185	.4955	10.8378	4.00	8.00
Jun	3	5.0000	2.64575	1.52753	-1.5724	11.5724	3.00	8.00
Jul	3	2.0000	1.00000	.57735	-.4841	4.4841	1.00	3.00
Aug	3	4.3333	4.16333	2.40370	-6.0090	14.6756	1.00	9.00
Sept	3	3.3333	1.52753	.88192	-.4612	7.1279	2.00	5.00
Oct	3	2.0000	1.00000	.57735	-.4841	4.4841	1.00	3.00
Total	36	3.9444	1.98486	.33081	3.2729	4.6160	1.00	9.00

ANOVA

Abundance of *Anopheles moucheti*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.556	11	4.596	1.263	.303
Within Groups	87.333	24	3.639		
Total	137.889	35			

Appendix 15: ANOVA comparing *Anopheles moucheti* abundance between the dry and the wet seasons.

```
ONEWAY An. moucheti BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles moucheti*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	41.4000	14.72413	6.58483	23.1176	59.6824	24.00	64.00
Wet season	7	43.7143	13.04753	4.93150	31.6473	55.7812	28.00	62.00
Total	12	42.7500	13.15727	3.79818	34.3903	51.1097	24.00	64.00

ANOVA

Abundance of *Anopheles moucheti*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.621	1	15.621	.083	.780
Within Groups	1888.629	10	188.863		
Total	1904.250	11			

Appendix 16: Monthly abundance of *Anopheles nili* collected from the study area

```
GET
  FILE='C:\Users\PC\Desktop\anopheles population by month town and
species.sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
ONEWAY Population BY Month
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles nili*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Nov	3	3.0000	2.64575	1.52753	-3.5724	9.5724	1.00	6.00
Dec	3	3.3333	3.21455	1.85592	-4.6521	11.3187	1.00	7.00
Jan	3	5.6667	1.52753	.88192	1.8721	9.4612	4.00	7.00
Feb	3	5.6667	4.16333	2.40370	-4.6756	16.0090	1.00	9.00
Mar	3	2.6667	2.51661	1.45297	-3.5849	8.9183	.00	5.00
Apr	3	6.6667	5.50757	3.17980	-7.0149	20.3482	3.00	13.00
May	3	7.0000	3.60555	2.08167	-1.9567	15.9567	4.00	11.00
Jun	3	5.0000	4.35890	2.51661	-5.8281	15.8281	2.00	10.00
Jul	3	8.3333	4.16333	2.40370	-2.0090	18.6756	5.00	13.00
Aug	3	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
Sept	3	2.3333	2.30940	1.33333	-3.4035	8.0702	1.00	5.00
Oct	3	7.3333	.57735	.33333	5.8991	8.7676	7.00	8.00
Total	36	5.0000	3.38062	.56344	3.8562	6.1438	.00	13.00

ANOVA

Abundance of *Anopheles nili*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	142.667	11	12.970	1.210	.333
Within Groups	257.333	24	10.722		
Total	400.000	35			

Appendix 17: ANOVA comparing *Anopheles nili* abundance between the dry and the wet seasons.

```
ONEWAY An. nili BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of *Anopheles nili*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	32.8000	17.89413	8.00250	10.5815	55.0185	19.00	59.00
Wet season	7	39.5714	13.79441	5.21380	26.8137	52.3291	27.00	60.00
Total	12	36.7500	15.24422	4.40063	27.0643	46.4357	19.00	60.00

ANOVA

Abundance of *Anopheles nili*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	133.736	1	133.736	.552	.475
Within Groups	2422.514	10	242.251		
Total	2556.250	11			

Appendix 18: Distribution of *Anopheles gambiae* s. s. larvae in the various breeding sites

```
ONEWAY Anopheles gambiae s. s. BY Breedingsites
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Distribution of *Anopheles gambiae* s. s. larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Basin	12	8.5833	7.87930	2.27456	3.5771	13.5896	.00	24.00
Broken clay pot	12	22.7500	16.01775	4.62392	12.5728	32.9272	.00	51.00
Canoe	12	24.2500	8.34620	2.40934	18.9471	29.5529	14.00	42.00
Drainage channel	12	33.5833	24.06226	6.94618	18.2949	48.8718	.00	66.00
Head pan	12	.7500	2.59808	.75000	-.9007	2.4007	.00	9.00
Plastic drum / container	12	33.2500	14.94307	4.31369	23.7556	42.7444	13.00	60.00
Puddle	12	23.9167	22.42344	6.47309	9.6695	38.1638	.00	58.00
River bank	12	45.7500	10.98863	3.17214	38.7682	52.7318	30.00	63.00
Swamp	12	15.0000	14.98484	4.32575	5.4791	24.5209	.00	45.00
Total	108	23.0926	19.46650	1.87316	19.3793	26.8059	.00	66.00

ANOVA

Distribution *Anopheles gambiae* s. s. larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18047.074	8	2255.884	9.926	.000
Within Groups	22500.000	99	227.273		
Total	40547.074	107			

Post Hoc Tests

Distribution of *Anopheles gambiae* s. s. larvae

Student-Newman-Keuls[a]

Breeding sites of *Anopheles gambiae* s. s. larvae	N	Subset for alpha = 0.05			
		1	2	3	4
Head pan	12	.7500			
Basin	12	8.5833	8.5833		
Swamp	12	15.0000	15.0000		
Broken clay pot	12		22.7500	22.7500	
Puddle	12		23.9167	23.9167	
Canoe	12		24.2500	24.2500	
Plastic drum / container	12			33.2500	33.2500
Drainage channel	12			33.5833	33.5833
River bank	12				45.7500
Sig.		.058	.089	.403	.110

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 19: Distribution of *Anopheles funestus* in the various breeding sites

```
ONEWAY Anopheles funestus BY Breedingsites
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Distribution of *Anopheles funestus* larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Basin	12	.9167	3.17543	.91667	-1.1009	2.9342	.00	11.00
Broken clay pot	12	6.7500	5.70685	1.64743	3.1240	10.3760	.00	14.00
Canoe	12	7.7500	4.02549	1.16206	5.1923	10.3077	2.00	14.00
Drainage channel	12	12.4167	10.85825	3.13451	5.5177	19.3157	.00	31.00
Head pan	12	.7500	2.05050	.59193	-.5528	2.0528	.00	7.00
Plastic drum / container	12	11.9167	11.61080	3.35175	4.5395	19.2938	.00	34.00
Puddle	12	11.1667	10.34701	2.98692	4.5925	17.7408	.00	29.00
River bank	12	21.5833	7.76306	2.24100	16.6509	26.5157	10.00	34.00
Swamp	12	10.1667	9.99848	2.88631	3.8139	16.5194	.00	29.00
Total	108	9.2685	9.81188	.94415	7.3969	11.1402	.00	34.00

ANOVA

Distribution of *Anopheles funestus* larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3887.463	8	485.933	7.501	.000
Within Groups	6413.750	99	64.785		
Total	10301.213	107			

Post Hoc Tests

Distribution of *Anopheles funestus* larvae

Student-Newman-Keuls[a]

Breeding sites of *Anopheles funestus* larvae	N	Subset for alpha = 0.05		
		1	2	3
Head pan	12	.7500		
Basin	12	.9167		
Broken clay pot	12	6.7500	6.7500	
Canoe	12	7.7500	7.7500	
Swamp	12		10.1667	
Puddle	12		11.1667	
Plastic drum / container	12		11.9167	
Drainage channel	12		12.4167	
River bank	12			21.5833
Sig.		.151	.519	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 20: Distribution of *Anopheles moucheti* in the various breeding sites

```
ONEWAY Anopheles moucheti BY Breedingsites
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Distribution of *Anopheles moucheti* larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Broken clay pot	12	.5833	1.50504	.43447	-.3729	1.5396	.00	5.00
Canoe	12	2.2500	2.86436	.82687	.4301	4.0699	.00	8.00
Drainage channel	12	.3333	.77850	.22473	-.1613	.8280	.00	2.00
Plastic drum / container	12	.7500	2.05050	.59193	-.5528	2.0528	.00	7.00
River bank	12	17.0833	9.67150	2.79192	10.9384	23.2283	.00	34.00
Swamp	12	9.9167	11.98832	3.46073	2.2997	17.5337	.00	33.00
Total	72	5.1528	8.89795	1.04863	3.0619	7.2437	.00	34.00

ANOVA

Distribution of *Anopheles moucheti* larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2843.403	5	568.681	13.511	.000
Within Groups	2777.917	66	42.090		
Total	5621.319	71			

Post Hoc Tests

Distribution of *Anopheles moucheti* larvae

Student-Newman-Keuls[a]

Breeding sites of *Anopheles moucheti* larvae	N	Subset for alpha = 0.05: 1	2	3
Drainage channel	12	.3333		
Broken clay pot	12	.5833		
Plastic drum / container	12	.7500		
Canoe	12	2.2500		
Swamp	12		9.9167	
River bank	12			17.0833
Sig.		.887	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 21: Distribution of *Anopheles nili* in the various breeding sites

```
ONEWAY Anopheles nili BY Breedingsites
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Distribution of *Anopheles nili* larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Broken clay pot	12	.1667	.57735	.16667	-.2002	.5335	.00	2.00
Canoe	12	5.0000	6.06030	1.74946	1.1495	8.8505	.00	18.00
Drainage channel	12	2.5000	5.71282	1.64915	-1.1298	6.1298	.00	17.00
Plastic drum / container	12	.1667	.57735	.16667	-.2002	.5335	.00	2.00
River bank	12	7.8333	8.13336	2.34790	2.6656	13.0010	.00	24.00
Swamp	12	6.0833	8.06179	2.32724	.9611	11.2055	.00	20.00
Total	72	3.6250	6.30546	.74311	2.1433	5.1067	.00	24.00

ANOVA

Distribution of *Anopheles nili* larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	609.958	5	121.992	3.638	.006
Within Groups	2212.917	66	33.529		
Total	2822.875	71			

Post Hoc Tests

Distribution of *Anopheles nili* larvae

Student-Newman-Keuls[a]

Breeding sites of *Anopheles nili* larvae	N	Subset for alpha = 0.05: 1	2
Broken clay pot	12	.1667	
Plastic drum / container	12	.1667	
Drainage channel	12	2.5000	2.5000
Canoe	12	5.0000	5.0000
Swamp	12	6.0833	6.0833
River bank	12		7.8333
Sig.		.102	.119

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 22: Comparing the monthly abundance of different *Anopheles* species larvae.

```
ONEWAY Abundance BY Anopheles species larvae
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Abundance of *Anopheles* species larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Anopheles gambiae s. s.	12	207.8333	88.60929	25.57930	151.5337	264.1330	89.00	342.00
Anopheles funestus	12	83.4167	39.45414	11.38943	58.3487	108.4846	25.00	143.00
Anopheles moucheti	12	30.9167	10.89168	3.14416	23.9964	37.8369	15.00	49.00
Anopheles nili	12	21.7500	13.23992	3.82204	13.3378	30.1622	4.00	42.00
Total	48	85.9792	88.83058	12.82159	60.1855	111.7729	4.00	342.00

ANOVA

Abundance of *Anopheles* species larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	264147.229	3	88049.076	36.301	.000
Within Groups	106723.750	44	2425.540		
Total	370870.979	47			

Post Hoc Tests

Abundance of *Anopheles* species larvae

Student-Newman-Keuls[a]

Different *Anopheles* species collected from the study	N	Subset for alpha = 0.05		
		1	2	3
Anopheles nili	12	21.7500		
Anopheles moucheti	12	30.9167		
Anopheles funestus	12		83.4167	
Anopheles gambiae s. s.	12			207.8333
Sig.		.651	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 23: ANOVA comparing the seasonal abundance of *Anopheles* mosquito larvae

```
ONEWAY Anopheles mosquito BY LARVAESeason
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Population of *Anopheles* mosquitoes collected as larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Wet season	7	404.8571	106.49480	40.25125	306.3659	503.3484	180.00	516.00
Dry season	5	258.6000	139.59692	62.42964	85.2675	431.9325	149.00	501.00
Total	12	343.9167	137.63817	39.73272	256.4655	431.3678	149.00	516.00

ANOVA

Population of *Anopheles* mosquitoes collected as larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62390.860	1	62390.860	4.273	.066
Within Groups	145996.057	10	14599.606		
Total	208386.917	11			

Appendix 24: ANOVA comparing the *Anopheles* species larvae in dry season

```
ONEWAY Abundance BY Anopheles species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Number of *Anopheles* species larvae collected in the dry season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Anopheles gambiae s. s.	5	141.8000	74.26439	33.21205	49.5886	234.0114	89.00	273.00
Anopheles funestus	5	66.8000	44.13842	19.73930	11.9949	121.6051	30.00	143.00
Anopheles moucheti	5	29.4000	13.08816	5.85320	13.1489	45.6511	15.00	49.00
Anopheles nili	5	20.6000	17.16974	7.67854	-.7190	41.9190	4.00	42.00
Total	20	64.6500	63.83224	14.27332	34.7756	94.5244	4.00	273.00

ANOVA

Number of *Anopheles* species larvae collected in the dry season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45698.550	3	15232.850	7.684	.002
Within Groups	31718.000	16	1982.375		
Total	77416.550	19			

Post Hoc Tests

Number of *Anopheles* species larvae collected in the dry season

Student-Newman-Keuls[a]

Anopheles species larvae collected in the dry season	N	Subset for alpha = 0.05: 1	2
Anopheles nili	5	20.6000	
Anopheles moucheti	5	29.4000	
Anopheles funestus	5	66.8000	
Anopheles gambiae s. s.	5		141.8000
Sig.		.258	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix 25: ANOVA comparing the *Anopheles* species larvae in wet season

```
ONEWAY Abundance BY Anopheles species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Number of *Anopheles* species larvae collected in the wet season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Anopheles gambiae s. s.	7	255.0000	66.99751	25.32268	193.0376	316.9624	128.00	342.00
Anopheles funestus	7	95.2857	34.06716	12.87618	63.7788	126.7926	25.00	129.00
Anopheles moucheti	7	32.0000	10.00000	3.77964	22.7515	41.2485	21.00	45.00
Anopheles nili	7	22.5714	11.08839	4.19102	12.3164	32.8265	5.00	40.00
Total	28	101.2143	101.44451	19.17121	61.8782	140.5504	5.00	342.00

ANOVA

Number of *Anopheles* species larvae collected in the wet season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	242623.571	3	80874.524	55.090	.000
Within Groups	35233.143	24	1468.048		
Total	277856.714	27			

Post Hoc Tests

Number of *Anopheles* species larvae collected in the wet season

Student-Newman-Keuls[a]

Anopheles species larvae collected in the wet season	N	Subset for alpha = 0.05: 1	2	3
Anopheles nili	7	22.5714		
Anopheles moucheti	7	32.0000		
Anopheles funestus	7		95.2857	
Anopheles gambiae s. s.	7			255.0000
Sig.		.649	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Appendix 26: ANOVA comparing the seasonal abundance of *Anopheles gambiae* s. s.

```
ONEWAY An. gambiae s. s. LARVAE by Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Population of *An. gambiae* s. s. collected as larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Wet season	7	255.0000	66.99751	25.32268	193.0376	316.9624	128.00	342.00
Dry season	5	141.8000	74.26439	33.21205	49.5886	234.0114	89.00	273.00
Total	12	207.8333	88.60929	25.57930	151.5337	264.1330	89.00	342.00

ANOVA

Population of *An. gambiae* s. s. collected as larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37374.867	1	37374.867	7.629	.020
Within Groups	48992.800	10	4899.280		
Total	86367.667	11			

Appendix 27: ANOVA comparing the seasonal abundance of *Anopheles funestus*

```
ONEWAY An. funestus BY LARVAESeason
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Population of *An. funestus* collected as larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Wet season	7	95.2857	34.06716	12.87618	63.7788	126.7926	25.00	129.00
Dry season	5	66.8000	44.13842	19.73930	11.9949	121.6051	30.00	143.00
Total	12	83.4167	39.45414	11.38943	58.3487	108.4846	25.00	143.00

ANOVA

Population of *An. funestus* collected as larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2366.688	1	2366.688	1.604	.234
Within Groups	14756.229	10	1475.623		
Total	17122.917	11			

Appendix 28: ANOVA comparing the seasonal abundance of *Anopheles moucheti*

```
ONEWAY Anopheles moucheti BY LARVAESeason
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Population of *An. moucheti* collected as larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Wet season	7	32.0000	10.00000	3.77964	22.7515	41.2485	21.00	45.00
Dry season	5	29.4000	13.08816	5.85320	13.1489	45.6511	15.00	49.00
Total	12	30.9167	10.89168	3.14416	23.9964	37.8369	15.00	49.00

ANOVA

Population of *An. moucheti* collected as larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.717	1	19.717	.153	.704
Within Groups	1285.200	10	128.520		
Total	1304.917	11			

Appendix 29: ANOVA comparing the seasonal abundance of *Anopheles nili*

```
ONEWAY An. nili BY LARVAESeason
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Total Population of An. nili larvae

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Wet season	7	11.0000	4.08248	1.54303	7.2243	14.7757	6.00	18.00
Dry season	5	8.6000	4.27785	1.91311	3.2883	13.9117	5.00	15.00
Total	12	10.0000	4.15605	1.19975	7.3594	12.6406	5.00	18.00

ANOVA

Total Population of An. nili larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.800	1	16.800	.970	.348
Within Groups	173.200	10	17.320		
Total	190.000	11			

Appendix 30: FRIEDMAN TEST comparing the monthly productivity of *Anopheles* mosquito breeding habitat.

```
NPAR TESTS
  /FRIEDMAN=October November December January February March April May June
July August September
  /MISSING LISTWISE.
```

NPar Tests

Friedman Test

Ranks

	Mean Rank
Number of Anopheles species larvae collected in October	4.06
Number of Anopheles species larvae collected in November	4.39
Number of Anopheles species larvae collected in December	3.00
Number of Anopheles species larvae collected in January	5.67
Number of Anopheles species larvae collected in February	4.78
Number of Anopheles species larvae collected in March	9.11
Number of Anopheles species larvae collected in April	8.78
Number of Anopheles species larvae collected in May	6.44
Number of Anopheles species larvae collected in June	9.11
Number of Anopheles species larvae collected in July	7.56
Number of Anopheles species larvae collected in August	7.72
Number of Anopheles species larvae collected in September	7.39

Test Statistics[a]

N	9
Chi-Square	36.380
df	11
Asymp. Sig.	.000

a. Friedman Test

Appendix 31: FRIEDMAN TEST comparing the monthly productivity of *Anopheles gambiae* s. s. breeding habitat.

```
NPAR TESTS
  /FRIEDMAN=October November December January February March April May June
July August September
  /MISSING LISTWISE.
```

NPar Tests

Friedman Test

Ranks

	Mean Rank
Number of Anopheles gambiae s. s. larvae collected in October	4.89
Number of Anopheles gambiae s. s. larvae collected in November	4.50
Number of Anopheles gambiae s. s. larvae collected in December	3.56
Number of Anopheles gambiae s. s. larvae collected in January	4.39
Number of Anopheles gambiae s. s. larvae collected in February	4.33
Number of Anopheles gambiae s. s. larvae collected in March	8.00
Number of Anopheles gambiae s. s. larvae collected in April	8.00
Number of Anopheles gambiae s. s. larvae collected in May	7.17
Number of Anopheles gambiae s. s. larvae collected in June	10.00
Number of Anopheles gambiae s. s. larvae collected in July	7.39
Number of Anopheles gambiae s. s. larvae collected in August	8.28
Number of Anopheles gambiae s. s. larvae collected in September	7.50

Test Statistics[a]

N	9
Chi-Square	35.997
df	11
Asymp. Sig.	.000

a. Friedman Test

Appendix 32: FRIEDMAN TEST comparing the monthly productivity of *Anopheles funestus* breeding habitat.

```
NPAR TESTS
  /FRIEDMAN=October November December January February March April May June
July August September
  /MISSING LISTWISE.
```

NPar Tests

Friedman Test

Ranks

	Mean Rank
Number of Anopheles funestus larvae collected in October	3.83
Number of Anopheles funestus larvae collected in November	5.17
Number of Anopheles funestus larvae collected in December	4.28
Number of Anopheles funestus larvae collected in January	5.39
Number of Anopheles funestus larvae collected in February	5.72
Number of Anopheles funestus larvae collected in March	8.94
Number of Anopheles funestus larvae collected in April	9.11
Number of Anopheles funestus larvae collected in May	7.28
Number of Anopheles funestus larvae collected in June	6.67
Number of Anopheles funestus larvae collected in July	6.56
Number of Anopheles funestus larvae collected in August	7.00
Number of Anopheles funestus larvae collected in September	8.06

Test Statistics[a]

N	9
Chi-Square	26.476
df	11
Asymp. Sig.	.006

a. Friedman Test

Appendix 33: FRIEDMAN TEST comparing the monthly productivity of *Anopheles moucheti* breeding habitat.

```
NPAR TESTS
  /FRIEDMAN=October November December January February March April May June
July August September
  /MISSING LISTWISE.
```

NPar Tests

Friedman Test

Ranks

	Mean Rank
Number of Anopheles moucheti larvae collected in October	6.58
Number of Anopheles moucheti larvae collected in November	4.92
Number of Anopheles moucheti larvae collected in December	5.33
Number of Anopheles moucheti larvae collected in January	7.42
Number of Anopheles moucheti larvae collected in February	7.00
Number of Anopheles moucheti larvae collected in March	6.83
Number of Anopheles moucheti larvae collected in April	5.33
Number of Anopheles moucheti larvae collected in May	6.00
Number of Anopheles moucheti larvae collected in June	8.83
Number of Anopheles moucheti larvae collected in July	6.42
Number of Anopheles moucheti larvae collected in August	7.08
Number of Anopheles moucheti larvae collected in September	6.25

Test Statistics[a]

N	6
Chi-Square	8.645
df	11
Asymp. Sig.	.655

a. Friedman Test

Appendix 34: FRIEDMAN TEST comparing the monthly productivity of *Anopheles nili* breeding habitat.

```
NPAR TESTS
  /FRIEDMAN=October November December January February March April May June
July August September
  /MISSING LISTWISE.
```

NPar Tests

Friedman Test

Ranks

	Mean Rank
Number of Anopheles nili larvae collected in October	5.25
Number of Anopheles nili larvae collected in November	5.75
Number of Anopheles nili larvae collected in December	5.08
Number of Anopheles nili larvae collected in January	7.50
Number of Anopheles nili larvae collected in February	5.50
Number of Anopheles nili larvae collected in March	7.33
Number of Anopheles nili larvae collected in April	7.75
Number of Anopheles nili larvae collected in May	7.17
Number of Anopheles nili larvae collected in June	6.75
Number of Anopheles nili larvae collected in July	7.58
Number of Anopheles nili larvae collected in August	6.00
Number of Anopheles nili larvae collected in September	6.33

Test Statistics[a]

N	6
Chi-Square	7.609
df	11
Asymp. Sig.	.748

a. Friedman Test

Appendix 35: ANOVA comparing Indoor and outdoor abundance of adult *Anopheles* mosquitoes

```
ONEWAY Abundance BY Location
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of adult *Anopheles* mosquitoes

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Indoor	12	264.5833	76.70067	22.14158	215.8501	313.3166	147.00	440.00
Outdoor	12	73.2500	23.36713	6.74551	58.4032	88.0968	35.00	111.00
Total	24	168.9167	112.36003	22.93539	121.4712	216.3621	35.00	440.00

ANOVA

Abundance of adult *Anopheles* mosquitoes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	219650.667	1	219650.667	68.331	.000
Within Groups	70719.167	22	3214.508		
Total	290369.833	23			

Appendix 36: ANOVA comparing indoor abundance of different *Anopheles* species.

```
ONEWAY Indoor BY Anopheles species
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

ANOVA

Indoor abundance of different *Anopheles* species collected as adults from the study area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	358433.562	3	119477.854	102.619	.000
Within Groups	51228.417	44	1164.282		
Total	409661.979	47			

Post Hoc Tests

Indoor abundance of different *Anopheles* species collected as adults from the study area

Student-Newman-Keuls[a]

Different *Anopheles* species collected as adults from the study area	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	12	8.9167	
Anopheles nili	12	10.9167	
Anopheles funestus	12	29.5833	
Anopheles gambiae s. s	12		215.1667
Sig.		.308	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 37: ANOVA comparing abundance of *Anopheles* species outdoors

```
ONEWAY Outdoor BY Anophelesspecies
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

ANOVA

Outdoor abundance of different *Anopheles* species collected as adults from the study area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21849.896	3	7283.299	79.041	.000
Within Groups	4054.417	44	92.146		
Total	25904.313	47			

Post Hoc Tests

Outdoor abundance of different *Anopheles* species collected as adults from the study area

Student-Newman-Keuls[a]

Different Anopheles species collected as adults from the study area	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	12	2.9167	
Anopheles nili	12	4.0833	
Anopheles funestus	12	11.4167	
Anopheles gambiae s. s	12		54.8333
Sig.		.088	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 38: ANOVA comparing Dry season and wet season abundance of adult *Anopheles* mosquitoes

```
ONEWAY Abundance BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS.
```

Descriptives

Abundance of adult *Anopheles* mosquitoes

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	283.0000	61.91123	27.68754	206.1271	359.8729	203.00	358.00
Wet season	7	377.0000	84.43735	31.91432	298.9085	455.0915	273.00	539.00
Total	12	337.8333	87.32472	25.20847	282.3499	393.3168	203.00	539.00

ANOVA

Abundance of adult *Anopheles* mosquitoes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25771.667	1	25771.667	4.435	.061
Within Groups	58110.000	10	5811.000		
Total	83881.667	11			

Appendix 39: ANOVA comparing indoor abundance of different *Anopheles* species in the dry season.

```
ONEWAY Indoor BY Anopheles species
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

ANOVA

Indoor abundance of different *Anopheles* species collected as adults in the dry season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	102353.750	3	34117.917	36.487	.000
Within Groups	14961.200	16	935.075		
Total	117314.950	19			

Post Hoc Tests

Indoor abundance of different *Anopheles* species collected as adults in the dry season

Student-Newman-Keuls[a]

Different *Anopheles* species collected as adults from the study area	N	Subset for alpha = 0.05	
		1	2
Anopheles nili	5	8.6000	
Anopheles moucheti	5	9.0000	
Anopheles funestus	5	25.4000	
Anopheles gambiae s. s	5		178.8000
Sig.		.667	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix 40: ANOVA comparing indoor abundance of different *Anopheles* species in the wet season.

```
ONEWAY Indoor BY Anopheles species
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

ANOVA

Indoor abundance of different *Anopheles* species collected as adults in the wet season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	263689.571	3	87896.524	85.284	.000
Within Groups	24735.143	24	1030.631		
Total	288424.714	27			

Homogeneous Subsets

Indoor abundance of different *Anopheles* species collected as adults in the wet season

Student-Newman-Keuls[a]

Different *Anopheles* species collected as adults from the study area	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	7	8.8571	
Anopheles nili	7	12.5714	
Anopheles funestus	7	32.5714	
Anopheles gambiae s. s	7		241.1429
Sig.		.366	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Appendix 41: ANOVA comparing abundance of *Anopheles* species outdoors in the dry season

```
NEW FILE.
DATASET NAME DataSet1 WINDOW=FRONT.
DATASET ACTIVATE DataSet0.
ONEWAY Outdoor BY Anophelesspecies
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

ANOVA

Outdoor abundance of different *Anopheles* species collected as adults in the dry season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6377.800	3	2125.933	41.361	.000
Within Groups	822.400	16	51.400		
Total	7200.200	19			

Post Hoc Tests

Outdoor abundance of different *Anopheles* species collected as adults in the dry season

Student-Newman-Keuls[a]

Different *Anopheles* species collected as adults from the study area	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	5	3.0000	
Anopheles nili	5	3.6000	
Anopheles funestus	5	8.6000	
Anopheles gambiae s. s	5		46.0000
Sig.		.451	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix 42: ANOVA comparing abundance of *Anopheles* species outdoors in the wet season

```
ONEWAY Outdoor BY Anopheles species
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

ANOVA

Outdoor abundance of different *Anopheles* species collected as adults in the wet season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15899.821	3	5299.940	51.019	.000
Within Groups	2493.143	24	103.881		
Total	18392.964	27			

Homogeneous Subsets

Outdoor abundance of different *Anopheles* species collected as adults in the wet season

Student-Newman-Keuls[a]

Different *Anopheles* species collected as adults from the study area	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	7	2.8571	
Anopheles nili	7	4.4286	
Anopheles funestus	7	13.4286	
Anopheles gambiae s. s	7		61.1429
Sig.		.149	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Appendix 43: ANOVA comparing gonotrophic states of *Anopheles* mosquitoes

```
ONEWAY Anopheles species BY Gonotrophicstate
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* mosquitoes in different gonotrophic states

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Fed	4	52.6500	4.23202	2.11601	45.9159	59.3841	47.20	57.00
Gravid	4	7.1500	1.60104	.80052	4.6024	9.6976	5.60	8.90
Half gravid	4	14.8500	4.81975	2.40988	7.1807	22.5193	11.10	21.80
Unfed	4	25.3250	2.33720	1.16860	21.6060	29.0440	22.60	28.30
Total	16	24.9938	18.06025	4.51506	15.3701	34.6174	5.60	57.00

ANOVA

Proportion of *Anopheles* mosquitoes in different gonotrophic states

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4745.092	3	1581.697	128.683	.000
Within Groups	147.497	12	12.291		
Total	4892.589	15			

Post Hoc Tests

Proportion of *Anopheles* mosquitoes

Student-Newman-Keuls[a]

Gonotrophic states of *Anopheles* mosquitoes	N	Subset for alpha = 0.05			
		1	2	3	4
Gravid	4	7.1500			
Half gravid	4		14.8500		
Unfed	4			25.3250	
Fed	4				52.6500
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Appendix 44: Paired T test comparing the endophagic and exophagic indices of *Anopheles* mosquitoes.

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles* mosquitoes	66.6167	12	8.22865	2.37541
	Exopgagic index of *Anopheles* mosquitoes	33.3833	12	8.22865	2.37541

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles* mosquitoes & Exopgagic index of *Anopheles* mosquitoes	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper			
Pair 1	Endopgagic index of *Anopheles* mosquitoes - Exopgagic index of *Anopheles* mosquitoes	33.23333	16.45729	4.75081	22.77687	43.68980	6.995	11	.000

Appendix 45: Paired T test comparing the endophagic and exophagic indices of *Anopheles* mosquitoes during the dry season.

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles* mosquitoes during the dry season	67.3000	5	10.87382	4.86292
	Exopgagic index of *Anopheles* mosquitoes during the dry season	32.7000	5	10.87382	4.86292

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles* mosquitoes during the dry season & Exopgagic index of *Anopheles* mosquitoes during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles* mosquitoes during the dry season - Exopgagic index of *Anopheles* mosquitoes during the dry season	34.60000	21.74764	9.72584	7.59673	61.60327	3.558	4	.024

Appendix 46: Paired T test comparing the endophagic and exophagic indices of *Anopheles* mosquitoes during the wet season.

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)

  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles* mosquitoes during the wet season	66.1286	7	6.68150	2.52537
	Exopgagic index of *Anopheles* mosquitoes during the wet season	33.8714	7	6.68150	2.52537

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles* mosquitoes during the wet season & Exopgagic index of *Anopheles* mosquitoes during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Endopgagic index of *Anopheles* mosquitoes during the wet season - Exopgagic index of *Anopheles* mosquitoes during the wet season	32.25714	13.36299	5.05074	19.89844	44.61585	6.387	6	.001

Appendix 47: Paired T test comparing the endophagic and exophagic indices of *Anopheles gambiae* s. s.

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles gambiae* s.s .	68.2000	12	9.01171	2.60146
	Exopgagic index of *Anopheles gambiae* s.s .	31.8000	12	9.01171	2.60146

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . & Exopgagic index of *Anopheles gambiae* s.s .	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . - Exopgagic index of *Anopheles gambiae* s.s .	36.40000	18.02342	5.20291	24.94847	47.85153	6.996	11	.000

Appendix 48: Paired T test comparing the endophagic and exophagic indices of *Anopheles gambiae* s. s. during the dry season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . during the dry season	68.4000	5	11.75649	5.25766
	Exopgagic index of *Anopheles gambiae* s.s . during the dry season	31.6000	5	11.75649	5.25766

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . during the dry season & Exopgagic index of *Anopheles gambiae* s.s . during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . during the dry season - Exopgagic index of *Anopheles gambiae* s.s . during the dry season	36.80000	23.51298	10.51532	7.60479	65.99521	3.500	4	.025

Appendix 49: Paired T test comparing the endophagic and exophagic indices of *Anopheles gambiae* s. s. during the wet season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . during the wet season	68.0571	7	7.52902	2.84570
	Exopgagic index of *Anopheles gambiae* s.s . during the wet season	31.9429	7	7.52902	2.84570

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . during the wet season & Exopgagic index of *Anopheles gambiae* s.s . during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles gambiae* s.s . during the wet season - Exopgagic index of *Anopheles gambiae* s.s . during the wet season	36.11429	15.05805	5.69141	22.18792	50.04066	6.345	6	.001

Appendix 50: Paired T test comparing the endophagic and exophagic indices of *Anopheles funestus*.

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles funestus*	62.8500	12	9.95312	2.87322
	Exopgagic index of *Anopheles funestus*	37.1417	12	9.93730	2.86865

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles funestus* & Exopgagic index of *Anopheles funestus*	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Endopgagic index of *Anopheles funestus* - Exopgagic index of *Anopheles funestus*	25.70833	19.89040	5.74186	13.07057	38.34609	4.477	11	.001

Appendix 51: Paired T test comparing the endophagic and exophagic indices of *Anopheles funestus* during the dry season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles funestus* during the dry season	65.3000	5	7.07001	3.16180
	Exopgagic index of *Anopheles funestus* during the dry season	34.7000	5	7.07001	3.16180

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles funestus* during the dry season & Exopgagic index of *Anopheles funestus* during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Endopgagic index of *Anopheles funestus* during the dry season - Exopgagic index of *Anopheles funestus* during the dry season	30.60000	14.14001	6.32361	13.04285	48.15715	4.839	4	.008

Appendix 52: Paired T test comparing the endophagic and exophagic indices of *Anopheles funestus* during the wet season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles funestus* during the wet season	61.1000	7	11.82032	4.46766
	Exopgagic index of *Anopheles funestus* during the wet season	38.8857	7	11.79837	4.45936

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles funestus* during the wet season & Exopgagic index of *Anopheles funestus* during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Endopgagic index of *Anopheles funestus* during the wet season - Exopgagic index of *Anopheles funestus* during the wet season	22.21429	23.61867	8.92702	.37066	44.05791	2.488	6	.047

Appendix 53: Paired T test comparing the endophagic and exophagic indices of *Anopheles moucheti*

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles moucheti*	63.4667	12	21.36843	6.16853
	Exopgagic index of *Anopheles moucheti*	36.5333	12	21.36843	6.16853

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles moucheti* & Exopgagic index of *Anopheles moucheti*	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles moucheti* - Exopgagic index of *Anopheles moucheti*	26.93333	42.73686	12.33707	-.22037	54.08704	2.183	11	.052

Appendix 54: Paired T test comparing the endophagic and exophagic indices of *Anopheles moucheti* during the dry season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles moucheti* during the dry season	61.8800	5	14.06776	6.29130
	Exopgagic index of *Anopheles moucheti* during the dry season	38.1200	5	14.06776	6.29130

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles moucheti* during the dry season & Exopgagic index of *Anopheles moucheti* during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles moucheti* during the dry season - Exopgagic index of *Anopheles moucheti* during the dry season	23.76000	28.13553	12.58259	-11.17487	58.69487	1.888	4	.132

Appendix 55: Paired T test comparing the endophagic and exophagic indices of *Anopheles moucheti* during the wet season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles moucheti* during the wet season	64.6000	7	26.48748	10.01133
	Exopgagic index of *Anopheles moucheti* during the wet season	35.4000	7	26.48748	10.01133

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles moucheti* during the wet season & Exopgagic index of *Anopheles moucheti* during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles moucheti* during the wet season - Exopgagic index of *Anopheles moucheti* during the wet season	29.20000	52.97496	20.02265	-19.79367	78.19367	1.458	6	.195

Appendix 56: Paired T test comparing the endophagic and exophagic indices of *Anopheles nili*

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles nili*	55.6250	12	14.28662	4.12419
	Exopgagic index of *Anopheles nili*	44.3750	12	14.28662	4.12419

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles nili* & Exopgagic index of *Anopheles nili*	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Endopgagic index of *Anopheles nili* - Exopgagic index of *Anopheles nili*	11.25000	28.57324	8.24838	-6.90457	29.40457	1.364	11	.200

Appendix 57: Paired T test comparing the endophagic and exophagic indices of *Anopheles nili* during the dry season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles nili* during the dry season	54.4000	5	18.48689	8.26759
	Exopgagic index of *Anopheles nili* during the dry season	45.6000	5	18.48689	8.26759

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles nili* during the dry season & Exopgagic index of *Anopheles nili* during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Endopgagic index of *Anopheles nili* during the dry season - Exopgagic index of *Anopheles nili* during the dry season	8.80000	36.97377	16.53517	-37.10900	54.70900	.532	4	.623

Appendix 58: Paired T test comparing the endophagic and exophagic indices of *Anopheles nili* during the wet season

```
T-TEST PAIRS=Indoor WITH Outdoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Endopgagic index of *Anopheles nili* during the wet season	56.5000	7	12.00875	4.53888
	Exopgagic index of *Anopheles nili* during the wet season	43.5000	7	12.00875	4.53888

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Endopgagic index of *Anopheles nili* during the wet season & Exopgagic index of *Anopheles nili* during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Endopgagic index of *Anopheles nili* during the wet season - Exopgagic index of *Anopheles nili* during the wet season	13.00000	24.01749	9.07776	-9.21248	35.21248	1.432	6	.202

Appendix 59: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles* mosquitoes.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors	61.5167	12	18.10815	5.22737
	Proportion of gravid *Anopheles* mosquitoes indoors	38.4833	12	18.10815	5.22737

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors & Proportion of gravid *Anopheles* mosquitoes indoors	12	-1.000	.000

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors - Proportion of gravid *Anopheles* mosquitoes indoors	23.03333	36.21630	10.45475	.02259	46.04407	2.203	11	.050

Appendix 60: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles* mosquitoes during the dry season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors during the dry season	67.2400	5	11.61348	5.19371
	Proportion of gravid *Anopheles* mosquitoes indoors during the dry season	32.7600	5	11.61348	5.19371

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors during the dry season & Proportion of gravid *Anopheles* mosquitoes indoors during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors during the dry season - Proportion of gravid *Anopheles* mosquitoes indoors during the dry season	34.48000	23.22697	10.38742	5.63991	63.32009	3.319	4	.029

Appendix 61: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles* mosquitoes during the wet season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors during the wet season	57.4286	7	21.55108	8.14554
	Proportion of gravid *Anopheles* mosquitoes indoors during the wet season	42.5714	7	21.55108	8.14554

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors during the wet season & Proportion of gravid *Anopheles* mosquitoes indoors during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference: Lower	95% Confidence Interval of the Difference: Upper			
Pair 1	Proportion of gravid *Anopheles* mosquitoes outdoors during the wet season - Proportion of gravid Anopheles mosquitoes indoors during the wet season	14.85714	43.10216	16.29109	-25.00571	54.71999	.912	6	.397

Appendix 62: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles gambiae* s. s.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors	62.3083	12	19.60364	5.65908
	Proportion of gravid *Anopheles gambiae* s. s. indoors	37.6917	12	19.60364	5.65908

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors & Proportion of gravid *Anopheles gambiae* s. s. indoors	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors - Proportion of gravid *Anopheles gambiae* s. s. indoors	24.61667	39.20728	11.31817	-.29445	49.52778	2.175	11	.052

Appendix 63: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles gambiae* s. s. during the dry season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors during the dry season	68.0000	5	12.04471	5.38656
	Proportion of gravid *Anopheles gambiae* s. s. indoors during the dry season	32.0000	5	12.04471	5.38656

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors during the dry season & Proportion of gravid *Anopheles gambiae* s. s. indoors during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors during the dry season - Proportion of gravid *Anopheles gambiae* s. s. indoors during the dry season	36.00000	24.08942	10.77311	6.08904	65.91096	3.342	4	.029

Appendix 64: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles gambiae* s. s. during the wet season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors during the wet season	58.2429	7	23.69725	8.95672
	Proportion of gravid *Anopheles gambiae* s. s. indoors during the wet season	41.7571	7	23.69725	8.95672

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors during the wet season & Proportion of gravid *Anopheles gambiae* s. s. indoors during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Proportion of gravid *Anopheles gambiae* s. s. outdoors during the wet season - Proportion of gravid *Anopheles gambiae* s. s. indoors during the wet season	16.48571	47.39449	17.91344	-27.34688	60.31831	.920	6	.393

Appendix 65: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles funestus*.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles funestus* outdoors	61.3250	12	14.19559	4.09791
	Proportion of gravid *Anopheles funestus* indoors	38.6750	12	14.19559	4.09791

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles funestus* outdoors & Proportion of gravid *Anopheles funestus* indoors	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper			
Pair 1	Proportion of gravid *Anopheles funestus* outdoors - Proportion of gravid *Anopheles funestus* indoors	22.65000	28.39118	8.19583	4.61110	40.68890	2.764	11	.018

Appendix 66: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles funestus* during the dry season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles funestus* outdoors during the dry season	67.8800	5	11.15200	4.98732
	Proportion of gravid *Anopheles funestus* indoors during the dry season	32.1200	5	11.15200	4.98732

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles funestus* outdoors during the dry season & Proportion of gravid *Anopheles funestus* indoors during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Proportion of gravid *Anopheles funestus* outdoors during the dry season - Proportion of gravid *Anopheles funestus* indoors during the dry season	35.76000	22.30399	9.97465	8.06594	63.45406	3.585	4	.023

Appendix 67: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles funestus* during the wet season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles funestus* outdoors during the wet season	56.6429	7	15.00498	5.67135
	Proportion of gravid *Anopheles funestus* indoors during the wet season	43.3571	7	15.00498	5.67135

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles funestus* outdoors during the wet season & Proportion of gravid *Anopheles funestus* indoors during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper			
Pair 1	Proportion of gravid *Anopheles funestus* outdoors during the wet season - Proportion of gravid *Anopheles funestus* indoors during the wet season	13.28571	30.00997	11.34270	-14.46888	41.04030	1.171	6	.286

Appendix 68: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles moucheti*.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors	42.5083	12	35.88001	10.35767
	Proportion of gravid *Anopheles moucheti* indoors	57.4917	12	35.88001	10.35767

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors & Proportion of gravid *Anopheles moucheti* indoors	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors - Proportion of gravid *Anopheles moucheti* indoors	-14.98333	71.76003	20.71534	-60.57748	30.61081	-.723	11	.485

Appendix 69: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles moucheti* during the dry season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors during the dry season	54.5200	5	33.82391	15.12651
	Proportion of gravid *Anopheles moucheti* indoors during the dry season	45.4800	5	33.82391	15.12651

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors during the dry season & Proportion of gravid *Anopheles moucheti* indoors during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors during the dry season - Proportion of gravid *Anopheles moucheti* indoors during the dry season	9.04000	67.64782	30.25303	-74.95587	93.03587	.299	4	.780

Appendix 70: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles moucheti* during the wet season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors during the wet season	33.9286	7	37.30106	14.09848
	Proportion of gravid *Anopheles moucheti* indoors during the wet season	66.0714	7	37.30106	14.09848

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors during the wet season & Proportion of gravid *Anopheles moucheti* indoors during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles moucheti* outdoors during the wet season - Proportion of gravid *Anopheles moucheti* indoors during the wet season	-32.14286	74.60212	28.19695	-101.13831	36.85260	-1.140	6	.298

Appendix 71: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles nili* during the dry season.

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles nili*	51.9667	12	27.10929	7.82578
	Proportion of gravid *Anopheles nili*	48.0333	12	27.10929	7.82578

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles nili* & Proportion of gravid *Anopheles nili*	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Proportion of gravid *Anopheles nili* - Proportion of gravid *Anopheles nili*	3.93333	54.21857	15.65155	-30.51550	38.38217	.251	11	.806

Appendix 72: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles nili* during the dry season

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles nili* during the dry season	65.4800	5	19.60872	8.76929
	Proportion of gravid *Anopheles nili* during the dry season	34.5200	5	19.60872	8.76929

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles nili* during the dry season & Proportion of gravid *Anopheles nili* during the dry season	5	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles nili* during the dry season - Proportion of gravid *Anopheles nili* during the dry season	30.96000	39.21745	17.53857	-17.73489	79.65489	1.765	4	.152

Appendix 73: Paired T test comparing the indoor and outdoor proportions of gravid *Anopheles nili* during the wet season

```
T-TEST PAIRS=Outdoor WITH Indoor (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Proportion of gravid *Anopheles nili* during the wet season	42.3143	7	28.81206	10.88993
	Proportion of gravid *Anopheles nili* during the wet season	57.6857	7	28.81206	10.88993

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Proportion of gravid *Anopheles nili* during the wet season & Proportion of gravid *Anopheles nili* during the wet season	7	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Proportion of gravid *Anopheles nili* during the wet season - Proportion of gravid *Anopheles nili* during the wet season	-15.37143	57.62412	21.77987	-68.66485	37.92199	-.706	6	.507

Appendix 74: ANOVA comparing the Human Blood Index of *Anopheles* species between the wet and the dry season.

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that fed on human blood.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	94.2000	1.72627	.77201	92.0566	96.3434	91.40	95.80
Wet season	7	95.2714	2.41296	.91201	93.0398	97.5030	91.30	99.10
Total	12	94.8250	2.13632	.61670	93.4676	96.1824	91.30	99.10

ANOVA

Proportion of *Anopheles* species that fed on human blood.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.348	1	3.348	.715	.418
Within Groups	46.854	10	4.685		
Total	50.202	11			

Appendix 75: ANOVA comparing the monthly Human Blood Index of *Anopheles* species

```
ONEWAY Proportion BY Months
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that fed on human blood.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
November	4	86.3250	16.52097	8.26049	60.0364	112.6136	66.70	100.00
December	4	81.8500	12.40228	6.20114	62.1152	101.5848	66.70	95.40
January	4	93.3000	7.07908	3.53954	82.0356	104.5644	83.30	100.00
February	4	76.8500	21.22616	10.61308	43.0744	110.6256	50.00	99.30
March	4	78.7000	13.68868	6.84434	56.9183	100.4817	66.70	97.80
April	4	86.4000	11.53632	5.76816	68.0431	104.7569	75.00	100.00
May	4	88.5000	13.09911	6.54955	67.6564	109.3436	71.40	100.00
June	4	88.6000	9.41913	4.70956	73.6121	103.5879	80.00	100.00
July	4	90.8500	8.37556	4.18778	77.5226	104.1774	83.30	100.00
August	4	79.1500	21.86237	10.93119	44.3621	113.9379	50.00	98.30
September	4	84.0000	31.00806	15.50403	34.6593	133.3407	37.50	100.00
October	4	87.3750	24.91778	12.45889	47.7252	127.0248	50.00	100.00
Total	48	85.1583	16.02568	2.31311	80.5050	89.8117	37.50	100.00

ANOVA

Proportion of *Anopheles* species that fed on human blood.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1154.542	11	104.958	.346	.968
Within Groups	10916.115	36	303.225		
Total	12070.657	47			

Appendix 76: ANOVA comparing the Human Blood Index of *Anopheles* species.

```
ONEWAY HBI BY Anopheles_species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that fed on human blood.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Anopheles gambiae s. s.	12	97.4667	2.32001	.66973	95.9926	98.9407	92.60	100.00
Anopheles funestus	12	86.7000	8.34070	2.40775	81.4006	91.9994	77.80	100.00
Anopheles moucheti	12	74.0083	23.36966	6.74624	59.1600	88.8567	37.50	100.00
Anopheles nili	12	82.4583	12.83762	3.70590	74.3017	90.6150	66.70	100.00
Total	48	85.1583	16.02568	2.31311	80.5050	89.8117	37.50	100.00

ANOVA

Proportion of *Anopheles* species that fed on human blood.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3425.812	3	1141.937	5.812	.002
Within Groups	8644.845	44	196.474		
Total	12070.657	47			

Post Hoc Tests

Proportion of *Anopheles* species that fed on human blood.

Student-Newman-Keuls[a]

Different *Anopheles* species	N	Subset for alpha = 0.05 1	2
Anopheles moucheti	12	74.0083	
Anopheles nili	12	82.4583	
Anopheles funestus	12	86.7000	86.7000
Anopheles gambiae s. s.	12		97.4667
Sig.		.079	.067

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 77: ANOVA comparing the Human Blood Index of *Anopheles* species in the dry season.

```
ONEWAY HBI BY Anopheles_species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that fed on human blood in the dry season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Anopheles gambiae s. s.	5	97.4800	2.27750	1.01853	94.6521	100.3079	94.90	100.00
Anopheles funestus	5	83.4000	7.41788	3.31738	74.1895	92.6105	77.80	95.00
Anopheles moucheti	5	75.1200	19.28748	8.62562	51.1714	99.0686	50.00	100.00
Anopheles nili	5	77.6200	14.23295	6.36517	59.9475	95.2925	66.70	100.00
Total	20	83.4050	14.58234	3.26071	76.5803	90.2297	50.00	100.00

ANOVA

Proportion of *Anopheles* species that fed on human blood in the dry season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1501.066	3	500.355	3.153	.054
Within Groups	2539.184	16	158.699		
Total	4040.250	19			

Appendix 78: ANOVA comparing the Human Blood Index of *Anopheles* species in the wet season.

```
ONEWAY HBI BY Anopheles_species
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that fed on human blood in the wet season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Anopheles gambiae s. s.	7	97.4571	2.53170	.95689	95.1157	99.7986	92.60	100.00
Anopheles funestus	7	89.0571	8.67753	3.27980	81.0318	97.0825	78.90	100.00
Anopheles moucheti	7	73.2143	27.41328	10.36125	47.8612	98.5673	37.50	100.00
Anopheles nili	7	85.9143	11.56063	4.36951	75.2225	96.6061	71.40	100.00
Total	28	86.4107	17.13239	3.23772	79.7675	93.0540	37.50	100.00

ANOVA

Proportion of *Anopheles* species that fed on human blood in the wet season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2123.935	3	707.978	2.929	.054
Within Groups	5801.071	24	241.711		
Total	7925.007	27			

Post Hoc Tests

Proportion of *Anopheles* species that fed on human blood in the wet season

Student-Newman-Keuls[a]

Different *Anopheles* species collected	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	7	73.2143	
Anopheles nili	7	85.9143	85.9143
Anopheles funestus	7	89.0571	89.0571
Anopheles gambiae s. s	7		97.4571
Sig.		.159	.362

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Appendix 79: ANOVA comparing seasonal Human Blood Index of *Anopheles gambiae* s. s.

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles gambiae* s. s that fed on human blood.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	97.4800	2.27750	1.01853	94.6521	100.3079	94.90	100.00
Wet season	7	97.4571	2.53170	.95689	95.1157	99.7986	92.60	100.00
Total	12	97.4667	2.32001	.66973	95.9926	98.9407	92.60	100.00

ANOVA

Proportion of *Anopheles gambiae* s. s that fed on human blood.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	1	.002	.000	.988
Within Groups	59.205	10	5.921		
Total	59.207	11			

Appendix 80: ANOVA comparing seasonal Human Blood Index of *Anopheles funestus*

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles funestus* that fed on human blood

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	83.4000	7.41788	3.31738	74.1895	92.6105	77.80	95.00
Wet season	7	89.0571	8.67753	3.27980	81.0318	97.0825	78.90	100.00
Total	12	86.7000	8.34070	2.40775	81.4006	91.9994	77.80	100.00

ANOVA

Proportion of *Anopheles funestus* that fed on human blood

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	93.343	1	93.343	1.389	.266
Within Groups	671.897	10	67.190		
Total	765.240	11			

Appendix 81: ANOVA comparing seasonal Human Blood Index of *Anopheles moucheti*

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles moucheti* that fed on human blood

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	75.1200	19.28748	8.62562	51.1714	99.0686	50.00	100.00
Wet season	7	73.2143	27.41328	10.36125	47.8612	98.5673	37.50	100.00
Total	12	74.0083	23.36966	6.74624	59.1600	88.8567	37.50	100.00

ANOVA

Proportion of *Anopheles moucheti* that fed on human blood

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.593	1	10.593	.018	.897
Within Groups	5996.957	10	599.696		
Total	6007.549	11			

Appendix 82: ANOVA comparing seasonal Human Blood Index of *Anopheles nili*

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles nili* that fed on human blood

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	77.6200	14.23295	6.36517	59.9475	95.2925	66.70	100.00
Wet season	7	85.9143	11.56063	4.36951	75.2225	96.6061	71.40	100.00
Total	12	82.4583	12.83762	3.70590	74.3017	90.6150	66.70	100.00

ANOVA

Proportion of *Anopheles nili* that fed on human blood

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	200.653	1	200.653	1.245	.291
Within Groups	1612.197	10	161.220		
Total	1812.849	11			

Appendix 83: ANOVA comparing the seasonal sporozoite rates of *Anopheles* mosquitoes

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* mosquitoes that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	3.1800	1.81025	.80957	.9323	5.4277	1.20	4.90
Wet season	7	2.1571	1.68311	.63616	.6005	3.7138	.60	5.60
Total	12	2.5833	1.73616	.50119	1.4802	3.6864	.60	5.60

ANOVA

Proportion of *Anopheles* mosquitoes that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.052	1	3.052	1.014	.338
Within Groups	30.105	10	3.011		
Total	33.157	11			

Appendix 84: ANOVA comparing the monthly sporozoite rates of *Anopheles* mosquitoes

```
ONEWAY Proportion BY Month
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* mosquitoes that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
November	4	.3750	.75000	.37500	-.8184	1.5684	.00	1.50
December	4	3.8750	4.50583	2.25291	-3.2948	11.0448	.00	8.40
January	4	7.2500	6.22709	3.11355	-2.6587	17.1587	.00	14.30
February	4	.3500	.70000	.35000	-.7639	1.4639	.00	1.40
March	4	2.6000	3.24243	1.62121	-2.5594	7.7594	.00	6.70
April	4	2.1000	3.16965	1.58482	-2.9436	7.1436	.00	6.70
May	4	1.7500	2.12368	1.06184	-1.6292	5.1292	.00	4.30
June	4	.1750	.35000	.17500	-.3819	.7319	.00	.70
July	4	1.7250	3.45000	1.72500	-3.7647	7.2147	.00	6.90
August	4	1.9750	3.43742	1.71871	-3.4947	7.4447	.00	7.10
September	4	1.3750	1.88746	.94373	-1.6284	4.3784	.00	4.00
October	4	1.5250	2.72687	1.36344	-2.8141	5.8641	.00	5.60
Total	48	2.0896	3.33685	.48163	1.1207	3.0585	.00	14.30

ANOVA

Proportion of *Anopheles* mosquitoes that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	163.202	11	14.837	1.483	.181
Within Groups	360.123	36	10.003		
Total	523.325	47			

Appendix 85: ANOVA comparing the sporozoite rates of different *Anopheles* species

```
ONEWAY Proportion BY Anophelesspecies
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Anopheles gambiae s. s.	12	2.8750	2.57227	.74255	1.2407	4.5093	.50	8.40
Anopheles funestus	12	3.4583	3.20410	.92495	1.4225	5.4941	.00	7.10
Anopheles moucheti	12	1.1917	4.12805	1.19167	-1.4312	3.8145	.00	14.30
Anopheles nili	12	.8333	2.88675	.83333	-1.0008	2.6675	.00	10.00
Total	48	2.0896	3.33685	.48163	1.1207	3.0585	.00	14.30

ANOVA

Proportion of *Anopheles* species that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58.497	3	19.499	1.846	.153
Within Groups	464.828	44	10.564		
Total	523.325	47			

Appendix 86: ANOVA comparing the sporozoite rates of different *Anopheles* species in the dry season.

```
ONEWAY Proportion BY Anophelesspecies
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that are infected with *Plasmodium* sporozoite in the dry season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Anopheles gambiae s. s.	5	3.9400	2.86932	1.28320	.3773	7.5027	1.40	8.40
Anopheles funestus	5	2.7600	3.78193	1.69133	-1.9359	7.4559	.00	7.10
Anopheles moucheti	5	2.8600	6.39515	2.86000	-5.0806	10.8006	.00	14.30
Anopheles nili	5	2.0000	4.47214	2.00000	-3.5529	7.5529	.00	10.00
Total	20	2.8900	4.25068	.95048	.9006	4.8794	.00	14.30

ANOVA

Proportion of *Anopheles* species that are infected with *Plasmodium* sporozoite in the dry season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.562	3	3.187	.153	.926
Within Groups	333.736	16	20.859		
Total	343.298	19			

Appendix 87: ANOVA comparing the sporozoite rates of different *Anopheles* species in the wet season

```
ONEWAY Proportion BY Anophelesspecies
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles* species that are infected with *Plasmodium* sporozoite in the wet season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Anopheles gambiae s. s.	7	2.1143	2.24085	.84696	.0418	4.1867	.50	6.90
Anopheles funestus	7	3.9571	2.93079	1.10773	1.2466	6.6677	.00	7.10
Anopheles moucheti	7	.0000	.00000	.00000	.0000	.0000	.00	.00
Anopheles nili	7	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	28	1.5179	2.41953	.45725	.5797	2.4561	.00	7.10

ANOVA

Proportion of *Anopheles* species that are infected with *Plasmodium* sporozoite in the wet season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.395	3	25.465	7.484	.001
Within Groups	81.666	24	3.403		
Total	158.061	27			

Post Hoc Tests

Proportion of Anopheles species that are infected with Plasmodium sporozoite in the wet season

Student-Newman-Keuls[a]

Different Anopheles species collected	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	7	.0000	
Anopheles nili	7	.0000	
Anopheles gambiae s. s.	7	2.1143	2.1143
Anopheles funestus	7		3.9571
Sig.		.102	.074

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 7.000.

Appendix 88: ANOVA comparing the seasonal sporozoite rates of *Anopheles gambiae* s. s.

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles gambiae* s. s. that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	3.9400	2.86932	1.28320	.3773	7.5027	1.40	8.40
Wet season	7	2.1143	2.24085	.84696	.0418	4.1867	.50	6.90
Total	12	2.8750	2.57227	.74255	1.2407	4.5093	.50	8.40

ANOVA

Proportion of *Anopheles gambiae* s. s. that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.722	1	9.722	1.542	.243
Within Groups	63.061	10	6.306		
Total	72.783	11			

Appendix 89: ANOVA comparing the seasonal sporozoite rates of *Anopheles funestus*

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles funestus* that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	2.7600	3.78193	1.69133	-1.9359	7.4559	.00	7.10
Wet season	7	3.9571	2.93079	1.10773	1.2466	6.6677	.00	7.10
Total	12	3.4583	3.20410	.92495	1.4225	5.4941	.00	7.10

ANOVA

Proportion of *Anopheles funestus* that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.180	1	4.180	.384	.549
Within Groups	108.749	10	10.875		
Total	112.929	11			

Appendix 90: ANOVA comparing the seasonal sporozoite rates of *Anopheles moucheti*

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles moucheti* that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	2.8600	6.39515	2.86000	-5.0806	10.8006	.00	14.30
Wet season	7	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	12	1.1917	4.12805	1.19167	-1.4312	3.8145	.00	14.30

ANOVA

Proportion of *Anopheles moucheti* that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23.857	1	23.857	1.458	.255
Within Groups	163.592	10	16.359		
Total	187.449	11			

Appendix 91: ANOVA comparing the seasonal sporozoite rates of *Anopheles nili*

```
ONEWAY Proportion BY Season
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Proportion of *Anopheles nili* that are infected with *Plasmodium* sporozoite

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	2.0000	4.47214	2.00000	-3.5529	7.5529	.00	10.00
Wet season	7	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	12	.8333	2.88675	.83333	-1.0008	2.6675	.00	10.00

ANOVA

Proportion of *Anopheles nili* that are infected with *Plasmodium* sporozoite

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.667	1	11.667	1.458	.255
Within Groups	80.000	10	8.000		
Total	91.667	11			

Appendix 92: ANOVA comparing Entomological Innoculation Rate of different *Anopheles* species.

```
ONEWAY EIR BY Anophelesspecies
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Entomological Innoculation Rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Anopheles gambiae s. s.	12	.10392	.080079	.023117	.05304	.15480	.027	.242
Anopheles funestus	12	.02925	.041449	.011965	.00291	.05559	.000	.149
Anopheles moucheti	12	.00242	.008372	.002417	-.00290	.00774	.000	.029
Anopheles nili	12	.00250	.008660	.002500	-.00300	.00800	.000	.030
Total	48	.03452	.060815	.008778	.01686	.05218	.000	.242

ANOVA

Entomological Innoculation Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.083	3	.028	13.339	.000
Within Groups	.091	44	.002		
Total	.174	47			

Post Hoc Tests

Entomological Innoculation Rate

Student-Newman-Keuls[a]

Different Anopheles species collected	N	Subset for alpha = 0.05	
		1	2
Anopheles moucheti	12	.00242	
Anopheles nili	12	.00250	
Anopheles funestus	12	.02925	
Anopheles gambiae s. s.	12		.10392
Sig.		.327	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Appendix 93: ANOVA comparing seasonal Entomological Innoculation Rate of *Anopheles gambiae* s. s.

```
ONEWAY EIR BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Entomological Innoculation Rate of *Anopheles gambiae* s. s .

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	.13200	.080486	.035994	.03206	.23194	.032	.212
Wet season	7	.08386	.079443	.030027	.01038	.15733	.027	.242
Total	12	.10392	.080079	.023117	.05304	.15480	.027	.242

ANOVA

Entomological Innoculation Rate of *Anopheles gambiae* s. s .

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	1	.007	1.060	.327
Within Groups	.064	10	.006		
Total	.071	11			

Appendix 94: ANOVA comparing seasonal Entomological Innoculation Rate of *Anopheles funestus*

```
ONEWAY EIR BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Entomological Innoculation Rate of *Anopheles funestus*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	.01520	.020861	.009330	-.01070	.04110	.000	.040
Wet season	7	.03929	.050770	.019189	-.00767	.08624	.000	.149
Total	12	.02925	.041449	.011965	.00291	.05559	.000	.149

ANOVA

Entomological Innoculation Rate of *Anopheles funestus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	1	.002	.983	.345
Within Groups	.017	10	.002		
Total	.019	11			

Appendix 95: ANOVA comparing seasonal Entomological Innoculation Rate of *Anopheles moucheti*

```
ONEWAY EIR BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Entomological Innoculation Rate of *Anopheles moucheti*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	.00580	.012969	.005800	-.01030	.02190	.000	.029
Wet season	7	.00000	.000000	.000000	.00000	.00000	.000	.000
Total	12	.00242	.008372	.002417	-.00290	.00774	.000	.029

ANOVA

Entomological Innoculation Rate of *Anopheles moucheti*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	1.458	.255
Within Groups	.001	10	.000		
Total	.001	11			

Appendix 96: ANOVA comparing seasonal Entomological Innoculation Rate of *Anopheles nili*

```
ONEWAY EIR BY Seasons
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Entomological Innoculation Rate of *Anopheles nili*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Dry season	5	.00600	.013416	.006000	-.01066	.02266	.000	.030
Wet season	7	.00000	.000000	.000000	.00000	.00000	.000	.000
Total	12	.00250	.008660	.002500	-.00300	.00800	.000	.030

ANOVA

Entomological Innoculation Rate of *Anopheles nili*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	1.458	.255
Within Groups	.001	10	.000		
Total	.001	11			

Appendix 97: Chisquare analysis for seasonal prevalence of malaria parasites.

```
CROSSTABS
  /TABLES=Seasons BY Prevalence
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Seasons of the year * Prevalence of malaria	5400	100.0%	0	0.0%	5400	100.0%

Seasons of the year * Prevalence of malaria Crosstabulation

			Prevalence of malaria		
			Positive	Negative	Total
Seasons of the year	Dry season	Count	1245	1005	2250
		Expected Count	1377.5	872.5	2250.0
	Wet season	Count	2061	1089	3150
		Expected Count	1928.5	1221.5	3150.0
Total		Count	3306	2094	5400
		Expected Count	3306.0	2094.0	5400.0

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	56.343[a]	1	.000		
Continuity Correction[b]	55.919	1	.000		
Likelihood Ratio	56.173	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	56.333	1	.000		
N of Valid Cases	5400				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 872.50.

b. Computed only for a 2x2 table

Appendix 98: Chisquare analysis for monthly prevalence of malaria parasites.

```
CROSSTABS
  /TABLES=Months BY Prevalence
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.


[DataSet0] C:\Users\PC\Desktop\MR. EGBUCHE\DOCUMENT\PHD DATA FOR PREVALENCE
ANALYSIS.sav
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Different months within the study period * Prevalence of malaria throughout the study period	5400	100.0%	0	0.0%	5400	100.0%

Different months within the study period * Prevalence of malaria throughout the study period Crosstabulation

			Prevalence of malaria throughout the study period		Total
			Positive	Negative	
Different months within the study period	November	Count	213	237	450
		Expected Count	275.5	174.5	450.0
	December	Count	243	207	450
		Expected Count	275.5	174.5	450.0
	January	Count	282	168	450
		Expected Count	275.5	174.5	450.0
	February	Count	259	191	450
		Expected Count	275.5	174.5	450.0
	March	Count	248	202	450
		Expected Count	275.5	174.5	450.0
	April	Count	372	78	450
		Expected Count	275.5	174.5	450.0
	May	Count	300	150	450
		Expected Count	275.5	174.5	450.0
	June	Count	306	144	450

		Expected Count	275.5	174.5	450.0
	July	Count	315	135	450
		Expected Count	275.5	174.5	450.0
	August	Count	259	191	450
		Expected Count	275.5	174.5	450.0
	September	Count	246	204	450
		Expected Count	275.5	174.5	450.0
	October	Count	263	187	450
		Expected Count	275.5	174.5	450.0
Total		Count	3306	2094	5400
		Expected Count	3306.0	2094.0	5400.0

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	184.728[a]	11	.000
Likelihood Ratio	194.654	11	.000
Linear-by-Linear Association	10.419	1	.001
N of Valid Cases	5400		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 174.50.

Appendix 99: Chisquare analysis for monthly prevalence of malaria parasites in the dry season.

```
CROSSTABS   /TABLES=Months BY Prevalence   /FORMAT=AVALUE TABLES
/STATISTICS=CHISQ   /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Different months within the study period * Prevalence of malaria in the dry season	2250	100.0%	0	0.0%	2250	100.0%

Different months within the study period * Prevalence of malaria in the dry season Crosstabulation

			Prevalence of malaria in the dry season		Total
			Positive	Negative	
Different months within the study period	November	Count	213	237	450
		Expected Count	249.0	201.0	450.0
	December	Count	243	207	450
		Expected Count	249.0	201.0	450.0
	January	Count	282	168	450
		Expected Count	249.0	201.0	450.0
	February	Count	259	191	450
		Expected Count	249.0	201.0	450.0
	March	Count	248	202	450
		Expected Count	249.0	201.0	450.0
Total		Count	1245	1005	2250
		Expected Count	1245.0	1005.0	2250.0

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	22.676[a]	4	.000
Likelihood Ratio	22.738	4	.000
Linear-by-Linear Association	6.647	1	.010
N of Valid Cases	2250		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 201.00.

Appendix 100: Chisquare analysis for monthly prevalence of malaria parasites in the wet season.

```
CROSSTABS
  /TABLES=Months BY Prevalence
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Different months within the study period * Prevalence of malaria in the wet season	3150	100.0%	0	0.0%	3150	100.0%

Different months within the study period * Prevalence of malaria in the wet season Crosstabulation

			Prevalence of malaria in the wet season		Total
			Positive	Negative	
Different months within the study period	April	Count	372	78	450
		Expected Count	294.4	155.6	450.0
	May	Count	300	150	450
		Expected Count	294.4	155.6	450.0
	June	Count	306	144	450
		Expected Count	294.4	155.6	450.0
	July	Count	315	135	450
		Expected Count	294.4	155.6	450.0
	August	Count	259	191	450
		Expected Count	294.4	155.6	450.0
	September	Count	246	204	450
		Expected Count	294.4	155.6	450.0
	October	Count	263	187	450
		Expected Count	294.4	155.6	450.0
Total		Count	2061	1089	3150
		Expected Count	2061.0	1089.0	3150.0

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	109.971[a]	6	.000
Likelihood Ratio	115.743	6	.000
Linear-by-Linear Association	81.489	1	.000
N of Valid Cases	3150		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 155.57.

Appendix 101: Chisquare analysis for prevalence of malaria parasites by communities in the study area

```
CROSSTABS
  /TABLES=Location BY Prevalence
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Locations in the study area * Prevalence of malaria (%) from Oct 16 to Sept 17	5400	100.0%	0	0.0%	5400	100.0%

Locations in the study area * Prevalence of malaria (%) from Oct 16 to Sept 17 Crosstabulation

Count

		Prevalence of malaria (%) from Oct 16 to Sept 17		Total
		Positive	Negative	
Locations in the study area	Aguleri	1116	684	1800
	Igbariam	1098	702	1800
	Nsugbe	1092	708	1800
Total		3306	2094	5400

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.730[a]	2	.694
Likelihood Ratio	.731	2	.694
Linear-by-Linear Association	.674	1	.412
N of Valid Cases	5400		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 698.00.

Appendix 102: Chi square analysis to compare the prevalence of malaria parasite by gender

```
CROSSTABS
  /TABLES=Gender BY Prevalence
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT
  /COUNT ROUND CELL.
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Gender of the study participants * Prevalence of malaria (%) from Oct 16 to Sept 17	5400	100.0%	0	0.0%	5400	100.0%

Gender of the study participants * Prevalence of malaria (%) from Oct 16 to Sept 17 Crosstabulation

Count

		Prevalence of malaria (%) from Oct 16 to Sept 17		Total
		Positive	Negative	
Gender of the study participants	Male	1756	1096	2852
	Female	1550	998	2548
Total		3306	2094	5400

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.309[a]	1	.578		
Continuity Correction[b]	.279	1	.597		
Likelihood Ratio	.309	1	.578		
Fisher's Exact Test				.595	.299
Linear-by-Linear Association	.309	1	.578		
N of Valid Cases	5400				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 988.06.

b. Computed only for a 2x2 table

Appendix 103: Chi square analysis to compare the prevalence of malaria parasite by Age group

```
CROSSTABS
  /TABLES=Age BY Prevalence   /FORMAT=AVALUE TABLES   /STATISTICS=CHISQ
  /CELLS=COUNT   /COUNT ROUND CELL.
```

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Age groups of the study participants * Prevalence of malaria (%) from Oct 16 to Sept 17	5400	100.0%	0	0.0%	5400	100.0%

Age groups of the study participants * Prevalence of malaria (%) from Oct 16 to Sept 17 Crosstabulation

Count

		Prevalence of malaria (%) from Oct 16 to Sept 17		
		Positive	Negative	Total
Age groups of the study participants	0 - 5 years	255	196	451
	6 - 15 years	379	261	640
	16 - 25 years	651	386	1037
	26 - 35 years	584	359	943
	36 - 45 years	472	312	784
	46 - 55 years	329	206	535
	56 - 65 years	353	209	562
	66 years and above	283	165	448
Total		3306	2094	5400

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	8.173[a]	7	.318
Likelihood Ratio	8.125	7	.322
Linear-by-Linear Association	3.167	1	.075
N of Valid Cases	5400		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 173.72.

Appendix 104: T – test comparing the mean malaria parasite intensity between the wet and the dry season.

```
T-TEST GROUPS=Season(1 2)
  /MISSING=ANALYSIS
  /VARIABLES=Intensity
  /CRITERIA=CI(.95).
```

T-Test

Group Statistics

	Seasons of the year	N	Mean	Std. Deviation	Std. Error Mean
Malaria parasite intensity	dry season	1245	298.4578	182.81656	5.18121
	Wet season	2061	242.2513	183.21926	4.03582

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Malaria parasite intensity	Equal variances assumed	1.554	.213	8.554	3304	.000	56.20650	6.57112	43.32261	69.09038
	Equal variances not assumed			8.558	2627.425	.000	56.20650	6.56755	43.32840	69.08460

Appendix 105: ANOVA comparing the monthly intensity of malaria parasite infection.

```
ONEWAY Intensity BY Months
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Malaria parasite intensity

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
November	213	327.8873	190.32680	13.04098	302.1807	353.5939	40.00	760.00
December	243	310.8642	187.20341	12.00910	287.2085	334.5199	40.00	760.00
January	282	304.2908	176.78936	10.52765	283.5677	325.0139	40.00	720.00
February	259	269.4981	174.18064	10.82306	248.1853	290.8109	40.00	720.00
March	248	284.6371	183.42631	11.64758	261.6958	307.5783	40.00	760.00
April	372	269.0323	184.93717	9.58855	250.1775	287.8870	40.00	760.00
May	300	232.2000	172.28494	9.94688	212.6252	251.7748	40.00	720.00
June	306	214.3791	150.44341	8.60028	197.4557	231.3025	40.00	680.00
July	315	209.8413	153.65082	8.65724	192.8077	226.8748	40.00	720.00
August	259	221.6216	187.42356	11.64593	198.6884	244.5548	40.00	800.00
September	246	215.6098	210.36856	13.41261	189.1910	242.0285	40.00	960.00
October	263	332.3194	196.17803	12.09686	308.5000	356.1388	40.00	800.00
Total	3306	263.4180	185.05556	3.21848	257.1076	269.7284	40.00	960.00

ANOVA

Malaria parasite intensity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6232111.898	11	566555.627	17.450	.000
Within Groups	106949464.387	3294	32467.961		
Total	113181576.286	3305			

Post Hoc Tests

Malaria parasite intensity

Student-Newman-Keuls[a,b]

Months of the year	N	Subset for alpha = 0.05		
		1	2	3
July	315	209.8413		
June	306	214.3791		
September	246	215.6098		
August	259	221.6216		
May	300	232.2000		
April	372		269.0323	
February	259		269.4981	
March	248		284.6371	
January	282		304.2908	304.2908
December	243		310.8642	310.8642
November	213			327.8873
October	263			332.3194
Sig.		.601	.055	.270

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 270.016.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 106: ANOVA comparing the monthly intensity of malaria parasite infection in the dry season.

```
ONEWAY Intensity BY Months
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Malaria parasite intensity in the dry season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
November	213	327.8873	190.32680	13.04098	302.1807	353.5939	40.00	760.00
December	243	310.8642	187.20341	12.00910	287.2085	334.5199	40.00	760.00
January	282	304.2908	176.78936	10.52765	283.5677	325.0139	40.00	720.00
February	259	269.4981	174.18064	10.82306	248.1853	290.8109	40.00	720.00
March	248	284.6371	183.42631	11.64758	261.6958	307.5783	40.00	760.00
Total	1245	298.4578	182.81656	5.18121	288.2930	308.6227	40.00	760.00

ANOVA

Malaria parasite intensity in the dry season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	496060.978	4	124015.245	3.743	.005
Within Groups	41080778.058	1240	33129.660		
Total	41576839.036	1244			

Post Hoc Tests

Malaria parasite intensity in the dry season

Student-Newman-Keuls[a,b]

Months of the year	N	Subset for alpha = 0.05	
		1	2
February	259	269.4981	
March	248	284.6371	
January	282	304.2908	304.2908
December	243	310.8642	310.8642
November	213		327.8873
Sig.		.057	.320

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 246.921.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 107: ANOVA comparing the monthly intensity of malaria parasite infection in the wet season.

```
ONEWAY Intensity BY Months
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Malaria parasite intensity in the wet season

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
April	372	269.0323	184.93717	9.58855	250.1775	287.8870	40.00	760.00
May	300	232.2000	172.28494	9.94688	212.6252	251.7748	40.00	720.00
June	306	214.3791	150.44341	8.60028	197.4557	231.3025	40.00	680.00
July	315	209.8413	153.65082	8.65724	192.8077	226.8748	40.00	720.00
August	259	221.6216	187.42356	11.64593	198.6884	244.5548	40.00	800.00
September	246	215.6098	210.36856	13.41261	189.1910	242.0285	40.00	960.00
October	263	332.3194	196.17803	12.09686	308.5000	356.1388	40.00	800.00
Total	2061	242.2513	183.21926	4.03582	234.3366	250.1661	40.00	960.00

ANOVA

Malaria parasite intensity in the wet season

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3284067.480	6	547344.580	17.068	.000
Within Groups	65868686.329	2054	32068.494		
Total	69152753.809	2060			

Post Hoc Tests

Malaria parasite intensity in the wet season

Student-Newman-Keuls[a,b]

Months of the year	N	Subset for alpha = 0.05		
		1	2	3
July	315	209.8413		
June	306	214.3791		
September	246	215.6098		
August	259	221.6216		
May	300	232.2000		
April	372		269.0323	
October	263			332.3194
Sig.		.561	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 289.347.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 108: ANOVA comparing the mean intensity of malaria parasite infection in the communities.

```
ONEWAY Intensity BY Location
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Aguleri	1116	26.1039	18.88829	.56541	24.9946	27.2133	4.00	96.00
Igbariam	1098	26.1913	18.31045	.55258	25.1070	27.2755	4.00	80.00
Nsugbe	1092	26.7363	18.31510	.55424	25.6488	27.8238	4.00	88.00
Total	3306	26.3418	18.50556	.32185	25.7108	26.9728	4.00	96.00

ANOVA

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	257.940	2	128.970	.376	.686
Within Groups	1131557.823	3303	342.585		
Total	1131815.763	3305			

Post Hoc Tests - Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

Student-Newman-Keuls[a,b]

Locations in the study area	N	Subset for alpha = 0.05 1
Aguleri	1116	26.1039
Igbariam	1098	26.1913
Nsugbe	1092	26.7363
Sig.		.702

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 1101.906.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 109: ANOVA comparing the mean intensity of malaria parasite infection between males and females

```
ONEWAY Intensity BY Gender
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Male	1756	266.2984	187.23165	4.46804	257.5352	275.0616	40.00	880.00
Female	1550	260.1548	182.56434	4.63714	251.0591	269.2506	40.00	960.00
Total	3306	263.4180	185.05556	3.21848	257.1076	269.7284	40.00	960.00

ANOVA

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31073.811	1	31073.811	.907	.341
Within Groups	113150502.474	3304	34246.520		
Total	113181576.286	3305			

Appendix 110: ANOVA comparing the mean intensity of malaria parasite infection among different age groups.

```
ONEWAY Intensity BY Age
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=SNK ALPHA(0.05).
```

Descriptives

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
0 - 5 years	255	270.6667	180.99231	11.33418	248.3457	292.9876	40.00	720.00
6 - 15 years	379	259.5778	175.52290	9.01601	241.8500	277.3056	40.00	720.00
16 - 25 years	651	274.3011	187.93025	7.36557	259.8379	288.7643	40.00	960.00
26 - 35 years	584	254.0925	189.57621	7.84472	238.6851	269.4998	40.00	800.00
36 - 45 years	472	265.8898	189.15404	8.70652	248.7814	282.9983	40.00	800.00
46 - 55 years	329	261.8237	180.41032	9.94634	242.2570	281.3904	40.00	760.00
56 - 65 years	353	260.1700	188.70665	10.04384	240.4165	279.9235	40.00	800.00
66 years and above	283	258.0212	179.45240	10.66734	237.0235	279.0189	40.00	760.00
Total	3306	263.4180	185.05556	3.21848	257.1076	269.7284	40.00	960.00

ANOVA

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	162567.448	7	23223.921	.678	.691
Within Groups	113019008.837	3298	34268.954		
Total	113181576.286	3305			

Post Hoc Tests

Intensity of malaria (number of parasites per µl of blood) from Oct 16 to Sept 17

Student-Newman-Keuls[a,b]

Age groups of the study participants	N	Subset for alpha = 0.05
		1
26 - 35 years	584	254.0925
66 years and above	283	258.0212
6 - 15 years	379	259.5778
56 - 65 years	353	260.1700
46 - 55 years	329	261.8237
36 - 45 years	472	265.8898
0 - 5 years	255	270.6667
16 - 25 years	651	274.3011
Sig.		.811

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 375.004.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 111: Breeding Habitats of *Anopheles* mosquitoes in Anambra East Local Government Area.

Clay pot

Canoe

Drainage channel

Drainage channel

Head pan

Plastic container

Puddle

Puddle

River bank

River bank

Swamp

Appendix 112: Bench work at the Nigerian Institute of Medical Research (NIMR) Lagos

a.

b.

c.

d.

e.

f.

g.

h.

i.

j.

k.

l.

m.

a. Morphological identification of *Anopheles* species
b. Dissection of *Anopheles gambiae* s. l for molecular studies
c. Dissection of *Anopheles gambiae* s. l for molecular studies
d. Labelling of eppendorf tube containing separate body parts of *Anopheles gambiae* s. l.
e. Wings and legs of *An. gambiae* s. l. to be used for DNA extraction and PCR
f. Abdomen of *An. gambiae* s. l. to be used for Human Blood Index (HBI) determination
g. Head and thorax of *An. gambiae* s. l. to be used for Sporozoite Rate (SR) determination
h. Preparation of PCR master mix
i. Loading of tubes containing DNA extracted from *Anopheles gambiae* s. l. into the Thermocycler.
j. Loading of the PCR products (applicorns) + master mix + ficoll dye into the electrophoretic machine.
k. Regulation of the electrophoretic machine
l. Placing the agarose gel into the gel documentation system for viewing of bands.
m. Visual result of an ELISA procedure for Sporozoite Rate determination.