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Impact of climatic factors on entomological indices of *Anopheles* mosquitoes in Rivers State, Nigeria

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Abstract

This study observed the effects of climatic factors on entomological indices of *Anopheles* species causing malaria in Rivers State. Indoor resident mosquitoes were caught using pyrethrum spray catch technique, and were identified morphologically. Female *Anopheles* mosquitoes' salivary glands were assessed, stained in Giemsa and viewed using a compound microscope. The result showed that a total of 764 individual mosquitoes comprising 3 species: *An. gambiae* (*s.l*) 455 (59.55%), *An. funestus*, 224 (29.32%) and *An. coustani,* 85 (11.12%) were recorded. A total of 141 mosquitoes were infected with sporozoites giving an overall Sporozoite Rate (SR) of 18.82%. *Anopheles gambiae* (*s.l*) had the highest sporozoite rate (23.30%) followed by *An. coustani* (18.82%) and then *An. funestus* (8.48%). Man Biting Rate (MBR) in Odual was 7.81b/n/p and 12.22b/n/p in Port Harcourt; Entomological Inoculation Rate (EIR) was 161.71 ib/n/p and 206.76 ib/n/p in Odual and Port Harcourt respectively. The SR, MBR and EIR showed a positive linear correlation with humidity and rainfall but a negative correlation with temperature across the two stations.

Introduction

Parasite prevalence is a fundamental measure required to understand the temporal and spatial variations, and epidemiology of infectious diseases. The prevalence of malaria morbidity, and control depends on a number of underlying mechanisms such as demographics (age, density and sex) (Isaksson *et al* 2013), ecological (season, habitat quality and elevation; Rooyen *et al* 2013) and life-history traits (body mass and fitness) (Isaksson *et al* 2013; Rooyen *et al* 2013 and Matthews *et al* 2016). Again, seasonal dynamics and geographical distributions of vector borne parasites are strongly governed by seasonal changes in vector abundance (Ishtiaq *et al* 2017).

Mosquitoes are still considered as the most dangerous arthropodal animal because their females feed on human blood and transmit parasitic diseases, such as malaria and filariasis as well as other viral diseases such as dengue, yellow fever and zika (WHO 2024). They are estimated to transmit diseases to more than 700 million people annually and responsible for the death of about 1 in 17 people (Lamidi *et al* 2019). Effective transmission of mosquito-borne disease requires successful contact between female mosquitoes and their hosts (Xu *et al* 2014). Among the Culicidae Family, members of the genera *Aedes, Anopheles, Culex, and Mansonia* are best known for their role in transmitting debilitating diseases worldwide (Lamidi *et al* 2019).

There are 5 *Plasmodium* species causing malaria in humans: *Plasmodium falciparum, P. malariae, P. ovale, P. vivax,* and *P. knowlesi* (Sato 2021; Bannister and Sherman 2009). Among these species, *P. falciparum* and *P. vivax* pose the greatest threat and are responsible for most malaria-related deaths globally. However, *P. falciparum* is the most prevalent in Africa as well as South-Eastern Asia, Eastern Mediterranean and Western Pacific Regions. *Plasmodium. vivax* has a wider distribution than *P. falciparum* and predominates in many countries outside of Africa, especially the Americas (WHO 2016a). In certain regions, malaria prevalence is worsening as a result of environmental changes, civil disturbances, global warming, increasing travel and increasing drug resistance (Church 2004)

The endemicity of malaria in any region is determined by indigenous *Anopheles* mosquitoes' abundance, feeding and resting behaviours, and their *Plasmodium* infectivity among other factors (Hassan *et al* 2019; Center for Disease Control (CDC) 2015). Malaria transmission is heterogeneous and heavily dependent on the local climatic and ecological conditions. The intensity of transmission varies according to geography and seasonality with variations commonly observed at both the community and household levels (Bejon *et al* 2014; Kang *et al* 2018). Mosquito control relies mainly on large scale Indoor Residual Spraying (IRS) and bed net distribution, which are the recommended strategies by the World Health Organization (WHO 2019). With proper implementation, these approaches have been shown to be highly effective in reducing human-mosquito contact and burden of malaria (Burkot *et al* 2018).

Malaria remains one of the most important infectious diseases in the world, responsible for approximately 200 million cases and 500,000 deaths annually (WHO 2018), with the majority of deaths occurring in young children in sub-Saharan Africa (Aleshnick *et al* 2020) out of which approximately 30% occur in Nigeria (WHO 2016b). Malaria in pregnancy has adverse prenatal outcomes such as low birth weights, anaemia, still birth, spontaneous abortion and maternal and neonatal mortality with about 200,000 newborn deaths annually (WHO 2013; Akinleye *et al* 2009; Amon *et al* 2014). Over the past decades, mass roll-outs of effective malaria control tools have resulted in a significant reduction in malaria and death, as well as transmission rates in endemic countries. However, this decline has stalled in many of these countries, and in some, a reversal of these gains is observed (WHO 2018). Importantly, the frontline antimalarial drugs do not effectively clear gametocytes from infected people, emphasizing their unique biology compared to the asexual blood stage parasites (Ngotho *et al* 2019).

The risk of malaria infection is partly dependent on the proportion of mosquitoes as vectors that contain the infectious sporozoite stages of plasmodial parasites. Sporozoites can only be found in female mosquitoes, which have lived long enough for the parasites to complete their sporogonic development and migrate from oocysts in the mid gut wall to the salivary glands for onward transmission. (Cator *et al* 2014; Baer *et al* 2007). When an infectious female bites a human host and starts probing, sporozoites are injected with saliva before ingestion of blood. During feeding on a sugar source, saliva is mixed with sugar, initiating digestion, which continues in the crop (Brugman *et al* 2018).

Extant malaria control strategies globally revolve around data gathering on the parasitological, entomological and the vectorial dynamics of the disease. The determination of the sporozoite and entomological inoculation rates of indoor resident mosquitoes will enhance the understanding of the epidemiology of the disease and the development of cost-effective control measures of malaria in Nigeria. Parasitological and entomological studies have several important roles to play in malaria control, including the following: identification of the mosquito vectors responsible for transmission of the disease, provision of basic information on the habits and habitats of vector species for purposes of planning effective control measures, monitoring the impact of control measures (for example, by determining changes in vector population density, rates of infection, susceptibility of vectors to insecticides, and residual effects of insecticides on treated surfaces) and contributing to the investigation of problem areas where control measures prove unsuccessful. Environmental factors are significant in the epidemiology of diseases carried by vectors. For mosquitoes to complete their life cycles, the climatic and environmental factors must be right. The primary determinants of malaria risk include: temperature, forest cover, water bodies, and humidity (Mazher *et al* 2018). This study evaluated the impact of climatic factors on entomological indices in Odual and Port Harcourt, Nigeria.

Materials and methods

Research design

This study was a cross-sectional study in which mosquitoes were collected from bedrooms, and other sleeping places for parasitological and entomological analysis.

Study area

The study was carried out in Odual in Abua/Odual Local Government Area and Port Harcourt, in Port Harcourt City Local Government Area, both in River State. Odual lies within latitude 6.2968°N and longitude 4.4913E while Port Harcourt lies within coordinates 4.8472°N, 6.9746°E. Rivers State is a coastal state in the Niger-Delta Region of Nigeria. River State has a tropical rainforest climate, which is defined by a short dry season and a noticeable rainy season that begins in March and lasts until October with a respite that typically occurs in August (Rubel and Kottek 2010). Majority of the year have very consistent temperatures ranging from a minimum of 21-23ºC to a maximum of 28-33ºC. Subsistence farming and fishing are typical in many rural villages, and agriculture and industry have a strong position in the economy. Common industries include food production, oil and gas extraction, oil and gas servicing, road construction, and dredging (Lawal and Umeuduji 2017).

Ethical consideration

Advocacy visits were paid to the selected households, and verbal informed consent sort and obtained from community and family heads prior to indoor sample collections. Ethical permit was also obtained from the office of the Research and Ethic Committee, University of Port Harcourt.

Sampling techniques

Indoor mosquito collection was carried monthly from March 2022 to February 2023 in each of the sites, covering wet and dry seasons. Twelve houses were selected randomly from each location based on consent and individual samples were collected based on Household Compliant Index (HCI). Mosquitoes were collected indoors by Pyrethrum Spray Collection (PSC) from 6.00am to 10.30am, after the inhabitants have left the rooms. All foods and sensitive materials were properly covered, windows and doors closed and all openings that could allow escape of sprayed insecticide sealed, as health and safety precautionary measures before application of insecticides. Collected mosquitoes were kept in petri dishes, each for a room. Rooms were sprayed in clock-wise direction until a mist was formed. The petri dishes were covered with paper tapes, labelled to indicate the site and location, number of persons that slept in the room, and date of sampling. These samples were later taken to the laboratory of the Department of Animal and Environmental Biology, University of Port Harcourt for sorting and identification.

Climatic data collection

Mean rainfall, relative humidity, and temperature were obtained from the Nigeria Metrological Agency (NiMet) sub-station in the Port Harcourt.

Morphological identification and sorting of the mosquitoes

Anopheline mosquitoes were separated from other mosquito species according to the morphological characteristics of their maxillary palps, the patterns of spots on the wings, thorax and terminal abdominal segments, and scales of the legs using a dissecting microscope following the taxonomic keys of Al-Eryani *et al* (2016).

Estimation of infective (sporozoites) rate of *Anopheles* species

The wings of the female *Anopheles* mosquitoes were removed prior to dissection with a dissecting needle. The salivary glands were accessed when the heads of the mosquitoes were gently pulled away from the thoraxes into a drop of saline solution on a glass slide. The glands were then severed from the heads, and covered with a cover slip. The cover slips were then gently pressed to rupture the salivary glands, releasing their contents into designated drops of saline solutions. A drop of absolute alcohol was applied and left for one minute, after which a drop of Giemsa's stain was added and left for another 40 minutes as recommended by WHO (1975). After this, the prepared samples were examined for sporozoites using the compound microscope at ×100 objectives.

Calculation of entomological indices

Man-biting, sporozoite and entomological inoculation rates were calculated according to WHO guidelines (2013) as follows:

$$\text{Manbiting rate (MBR)} = \frac{\text{No.of } \textit{Anopheles} \text{ spp caught}}{\text{No.of occupants in rooms sampled}} \times 100 \ldots\ldots\ldots\ldots 1$$

$$\text{Sporozoite rate (SR)} = \frac{\text{No.of } \textit{Anopheles} \text{ spp infected}}{\text{Total No.of } \textit{Anopheles} \text{ spp examined}} \times 100 \ldots\ldots\ldots\ldots 2$$

$$\text{Entomological inoculation rate (EIR)} = \text{MBR} \times \text{SR} \ldots\ldots\ldots\ldots 3$$ (Ebenezer *et al* 2016)

Data analysis

The results of the study were analysed using frequencies and descriptive statistics such as means, standard deviations and percentages to describe the study population in relation to relevant variables. Linear regression was used to show the association between dependent and independent variables, and the differences were considered significant at $p < 0.05$.

Results

The abundance and distribution of the *Anopheles* mosquitoes in the study area showed that a total of 764 individuals comprising of 3 species; *An. gambiae* s.l, 455 (59.55%), *An. funestus*, 224 (29.32%) and *An. coustani,* 85(11.12%) were examined for sporozoites. Of the 764 samples examined, 141 were infected giving an overall SR of 18.45%. *An. gambiae* s.l had the highest SR (23.30%) followed by *An. coustani* (18.82%) while *An. funestus* had 8.48% as shown in Table 1. In Odual, the SR was 23.70%, 17.07% and 12.28% for *An. gambiae s.l.*, *An. funestus* and *An. Coustani,* respectively while the SR in Port Harcourt were 22.95%, 6.56% and 32.14% for *An. gambiae* s.l., *An. funestus* and *An. coustani* respectively (Table 2). The entomological indices indicated that MBR in Odual was 7.81b/n/p while that of Port Harcourt was 12.22b/n/p. The EIR were 161.71 ib/n/p and 206.76 ib/n/p for Odual and Port Harcourt, respectively (Table 3).

Table 1. Overall sporozoites rates of *Anopheles* mosquitoes in Rivers State

Mosquito species	No. examined	No. infected (%)	SR (%)
An. gambiae s.l	455	106 (%)	23.30
An. funestus	224	19	8.48
An. coustani	85	16	18.82
Total (%)	764	141	18.45

Table 2. *Anopheles* spp. sporozoite rates in Odual and Port Harcourt, Rivers State

Samples	Port Harcourt		Odual	
	No. exam.	SR(%)	No. exam.	SR(%)
An. gambiae s.l	244	56(22.95)	211	50(23.70)
An. funestus	183	12(6.56)	41	7(17.07)
An. coustani	28	9(32.14)	57	7(12.28)
Total	455	77(16.92)	309	64(20.71)

Table 3. Entomological indices in Odual and Port Harcourt, Rivers State

Locations (LGA)	MBR (b/n/p)	SR (%)	EIR (ib/n/p)
Odual	7.81	20.71	161.74
Port Harcourt	12.22	16.92	206.76
Total	10.02	18.82	188.58

MBR=Man Biting Rate; SR= Sporozoite Rate; EIR= Entomological Inoculation Rate (MBR ×SR)

The impact of climatic factors on entomological indices showed that in Odual, SR had a positive correlation with humidity with a linear regression equation of $y = 0.409x - 13.586$; and SR increases 0.4 times for every increase in humidity while humidity contributed 42.96% of the variation in SR (coefficient of determination, $R^2 = 0.4296$). In Port Harcourt, SR also had a positive correlation with humidity with a linear relation of $y = 1.0578x - 71.944$. The graphical analysis indicated that for every increase in humidity, SR increases 1.0578times, and humidity contributed 62.53% of the variation of in SR ($R^2 = 0.6253$) as shown in Figure 1.

The EIR value for Odual also had a positive correlation with humidity with a linear equation of $y = 1.1846x + 44.62$. The EIR increases 1.1846 times for every increase in humidity; and humidity contributed 47.73% for variations in EIR. In Port Harcourt, there was also a positive correlation between the EIR and humidity, with humidity accounting for 0.43% of changes in EIR (Figure 2).

Man Biting Rate (MBR) also had a positive linear correlation with humidity across the various locations with linear equations of y = 0.5069x - 29.35 and y = 0.1089x - 0.6951 for Port Harcourt and Odual respectively. With coefficients of determination (R^2) of 0.3543 and 0.0978, humidity contributed 35.43% and 9.78% to the variations in MBRs in Port Harcourt and Odual, respectively (Figure 3).

Figure 4 showed a positive correlation between MBR and rainfall in the study areas with linear regression equations of y = 0.0321x + 9.073 and y = 0.0063x + 7.625 for Odual and Port Harcourt, respectively. Coefficients of determination (R^2) were 0.5287 and 0.1007 for Odual and Port Harcourt respectively. Rainfall accounted for 52.87% and 10.07% of variations in MBR in Odual and Port Harcourt, respectively.

Sporozoite rates for Odual and Port Harcourt, again, revealed a linear relationship with rainfall; linear regression equations were y = 0.0065x + 19.595 and y = 0.0438x + 11.05, respectively and rainfall contributed 3.32% and 39.85% of the variations in SRs, respectively (Figure 5).

The impact of rainfall on EIR indicated that in Port Harcourt, there was a positive correlation between rainfall and EIR (y = 1.0603x + 102.24), and rainfall contributed 52% of the variation in the recorded EIR (R^2 = 0.52). There was a negative correlation between rainfall and EIR in Odual (y = -0.0287x + 147) with 0.09% of the variation in EIR being attributed by the rainfall (R^2 = 0.0009; Figure 6).

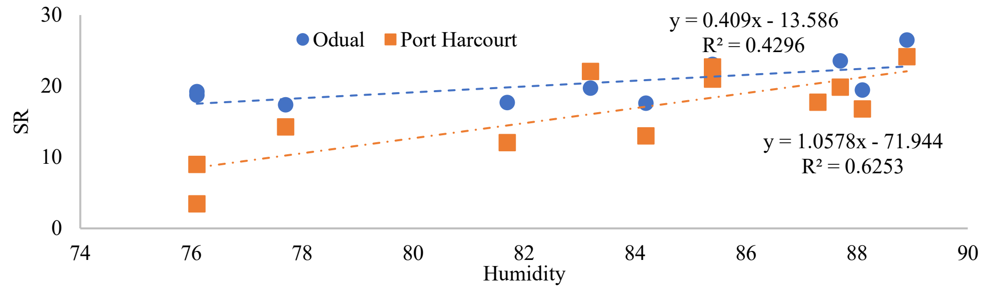

Figure 1. Linear relationship between various Sporozoite Rate (SR) and humidity

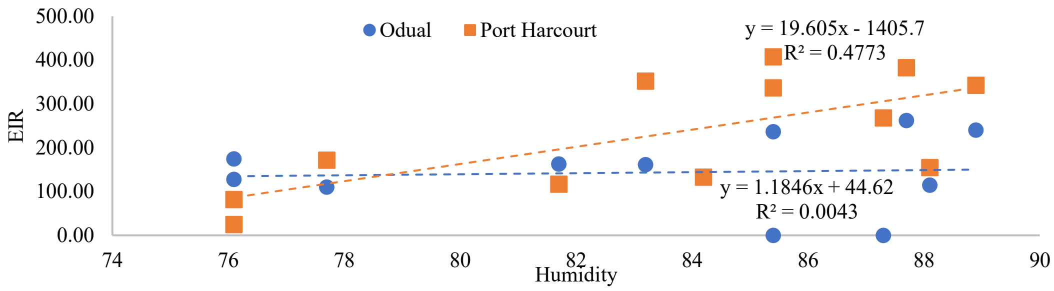

Figure 2. Linear relationship between Entomological Inoculation Rate (EIR) and humidity

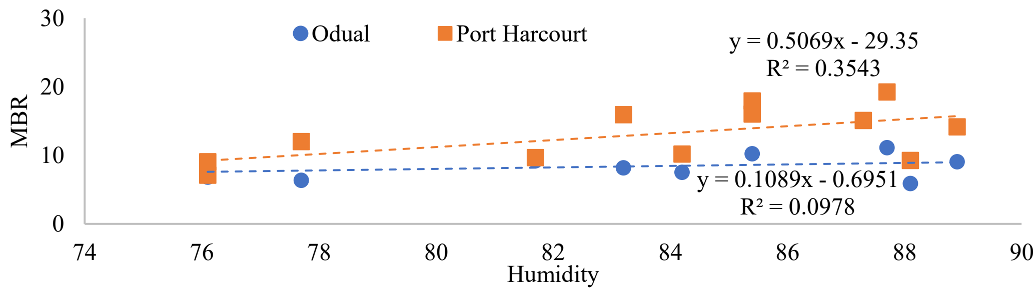

Figure 3. Linear relationship between Man Biting Rate (MBR) and humidity

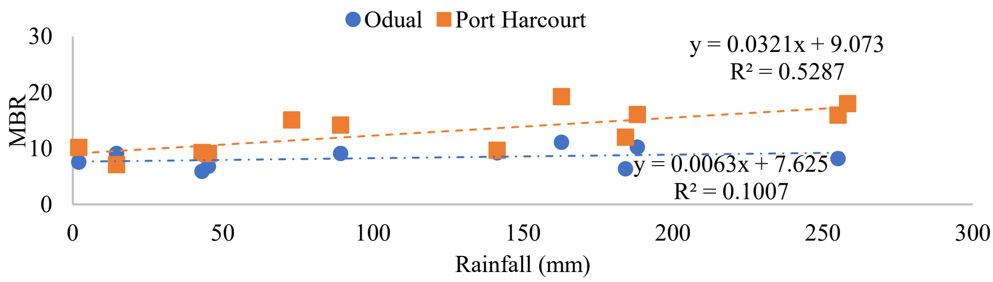

Figure 4. Linear relationship between Man Biting Rate (MBR) and rainfall

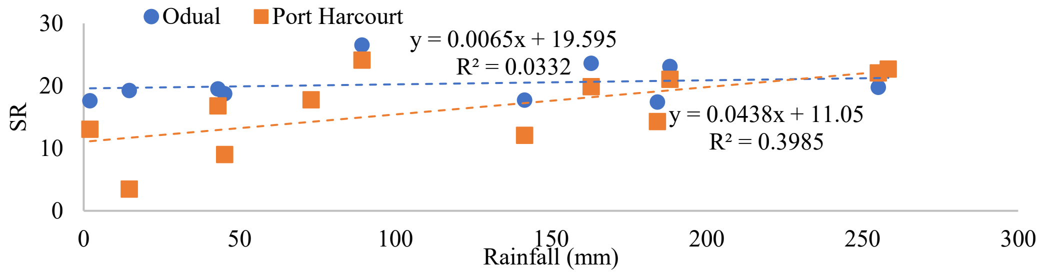

Figure 5. Linear relationship between Man Biting Rate (MBR) and rainfall

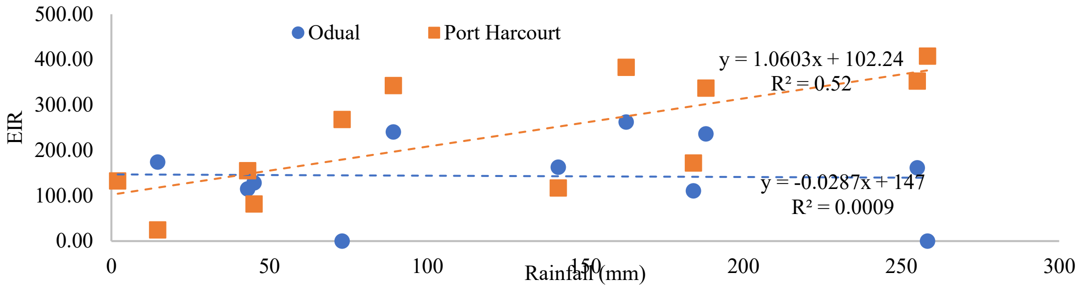

Figure 6. Linear regression between Entomological Inoculation Rate (EIR) and rainfall.

Temperature had a negative linear correlations with MBR in Odual (y =-0.6285x + 25.191) and Port Harcourt (y = -2.0505x + 67.565). It also contributed 13.66% and 27.69% to the MBRs respectively as shown in Figure 7. Similarly, temperature had negative linear correlations with SR in Odual (y = -2.2702x + 81.21) and Port Harcourt (y = -3.088x + 98.582), and contributed 55.49% and 25.46% to the variations in SR in both locations respectively (Figure 8). The effect of temperature on the EIR was skewed, having a positive correlation (y = 2.9601x + 64.702) in Odual and a negative correlation (y = -72.675x + 2166) in Port Harcourt (Figure 9).

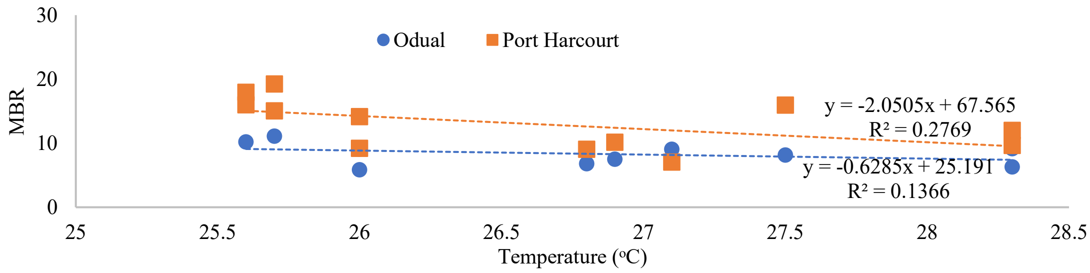

Figure 7. Linear relationship between Man Biting Rate (MBR) and temperature

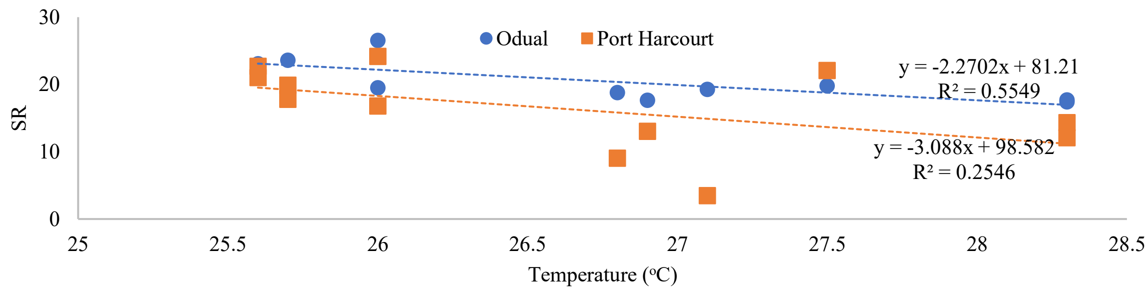

Figure 8. Linear relationship between Sporozoite Rate (SR) and temperature

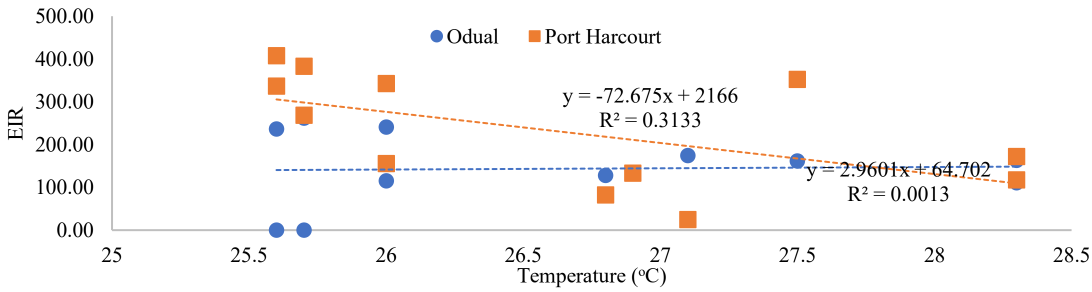

Figure 9. Linear relationship between Entomological Inoculation Rate (EIR) and temperature

Discussion

Entomological studies have several important roles to play in malaria control such as identification of the mosquito vectors responsible for transmission of the disease, provision of basic information on the habits and habitats of the vector species for purposes of planning effective control measures, monitoring the impact of control measures and contributing to the investigation of problem areas where control measures prove unsuccessful. In the present study, the overall sporozoite rate of 18.45% was higher than the 12.6% reported by Yohanna *et al* (2019) in Gombe State but lower than the 54.9% reported in Makurdi, Benue State by Msugh-Ter *et al* (2014), and 75% in Rivers State by Noutcha *et al* (2018). The sporozoite rates within the study area varied across the locations being higher in Odual (20.71%) than Port Harcourt (16.92%). The difference in sporozoite rates could be attributed to seasonality, geographical specificity of the areas, vegetation cover, abundance of *Anopheles* species examined, stage of *Plasmodium* development in the vector (Carter *et al* 2008), feeding and resting habits, host physiology and use of mosquito preventive measures (Aly *et al* 2009). Across the locations, SR showed positive linear correlation with rainfall and humidity but a negative correlation with temperature. This observation is at variance with the report of Adeola *et al* (2017) who asserted that temperature increases SR by reducing the incubation time of the parasites in mosquitoes. This implies that understanding the impact of temperature on SR can inform targeted interventions and predictive modeling for malaria control.

All the *Anopheles* mosquitoes caught during the study were known vectors of *Plasmodium*. In Port Harcourt, although *An. gambiae s.l.* was the most abundant, *An. coustani* had the highest SR. However, in Odual, *An. gambiae s.l.* had the highest abundance and SR. The emergence of *An. coustani* with the highest SR implies it has the potential to be to the key malaria vector if its population density increases in the region. This is an indication that, two main malaria vectors occurred in the zone. Ebenezer *et al* (2016) and Nyirakanani *et al* (2017) also reported *An. gambiae* s.l. as the major malaria vector in their studies in Bayelsa State, Nigeria, and southern Rwanda, respectively. *An. gambiae* s.l. had been reported to show both exophagic and endophilic behaviours (Degefa *et al* 2021), and thus, would require both indoor and outdoor control measures to combat malaria outbreaks caused by it. The higher abundance of *An. gambiae* s.l. in Odual and Port Harcourt could be due to the availability of favourable larvae breeding sites such as stagnant drainages, pits, floodplains and swamps that characterized the study areas.

The MBR in the present study (10.02 b/n/p) was low compared to 1129 and 2480 MBRs of *An. gambiae* and *An. gambiae* reported by Garrett-Jones and Shidrawi (1969) and Service (1965), respectively. The MBR in a location could be affected by factors such as crowded rooms, mosquitoes feeding and resting preferences and the gonotrophic cycle of the vector (Paaijmans and Thomas 2011). In the current study, MBR had a positive correlation with the rainfall and humidity, and a negative correlation with temperature. The MBRs also varied between 7.81b/n/p from 309 mosquitoes to 12.22b/n/p from 455 mosquitoes captured in Odual and Port Harcourt respectively. The MBRs are reported to be dependent on the number of adult female *Anopheles* species caught and the number of occupants in the room. Thus, factors such as rainfall, temperature and humidity that affect the abundance of adult mosquitoes (Jemal and Al-Thukair 2018) may have inadvertently affected MBR values of a particular species and zone. The use of insecticides can also adversely affect Anopheles abundance in a room; and migration can affect human population in an area. Both these circumstances affect MBRs.

There is no relevant or accessible literature on the impact of climatic factors on entomological indices as at time of this report hence, this observation could be further investigated to elucidate its health implications on the residents of the study areas. We therefore assume that rainfall increases availability of water bodies for larvae breeding, and subsequently increases the abundance of adult mosquitoes to influence the biting rates of the *Anopheles* mosquitoes. Again, the habitats for both the larvae and adult population densities are directly impacted by rainfall patterns and frequencies in reference to the report of Simon-Oke and Olofintoye (2015).

The impact of climatic factors on SR in the present study revealed that SR had a positive linear correlation with humidity and rainfall, but an inverse correlation with temperature. The SR is determined by the abundance of infected *Anopheles* mosquitoes in the room, and this abundance can be reduced through the use of indoor spraying of insecticides (Sarfraz *et al* 2019). The host preference and within-host plasmodial infection may also influence the SR level. This is because mosquitoes only get infected by taking blood meals from infected hosts (Isa *et al* 2015; Maria and Joaeo 2019).

Conclusion

In the evaluation of sporozoite rate in the study area, three major malaria vectors were recorded. *An. coustani* has the potential to be the main malaria vector in the region if it records a population increase. Results revealed that entomological indices varied across the locations, and climatic factors recorded a skewed influence on the EIR, MBR and SR. Understanding the impact of climatic factors on SR and MBR would help in predicting malaria outbreaks, developing effective control measures and improve malaria surveillance.

Conflict of Interest

Authors declare that no conflict of interest exist.

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