Ecology of Anopheles Mosquito Larvae in Different Ecological Zones in Ghana

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Abstract

Background: Understanding the ecology of larval malaria mosquitoes is important in a changing environment is important in developing effective control tools or programmes. This study characterized the breeding habitats of *Anopheles* mosquitoes in rural communities in different ecological zones in Ghana during the dry and rainy seasons.

Methods: The spatio-temporal distribution, species composition, and abundance of larval *Anopheles* mosquitoes in breeding habitats were studied in 3 ecological zones of Ghana. These were Anyakpor (Coastal Savanna area), Duase (Forest area), Libga, Pagazaa, and Kpalsogu (Sahel Savanna area). Larvae were collected using standard dippers and were raised in the insectary for identification.

Results: Out of a total of 7,984 mosquito larvae collected, 2,152 (27.26%) were Anophelines and were more abundant in the rainy season (70.82%) than in the dry season (29.18 %). The Anophelines were made up of 2,128 (98.88%) *An. gambiae* s.l., 16 (0.74%) *An. rufipes* and 8 (0.37%) *An. pharoensis*. In Anyakpor and Duase, dug-out wells were the most productive habitat in the dry (1.59 larvae/dip and 1.47 larvae/dip) and rainy seasons (11.28 larvae/dip and 2.05 larvae/dip). The most productive habitats in Kpalsogu were natural ponds in the dry season (0.89 larvae/dip) and swamps in the rainy season (2.57 larvae/dip). In Libga, the most productive habitats were drainage ditches in the dry season (0.30 larvae/dip) and furrows in the rainy season (1.83 larvae/dip). The most productive habitats in Pagazaa were puddles (1.44 larvae/dip). *Anopheles coluzzii* was the most abundant sibling species in all the ecological zones except Libga in the sahel savannah area where *An. gambiae* s.s was the most abundant. *Anopheles melas* and *An. arabiensis* were encountered only in the coastal savannah and the sahel savanna areas respectively. Larval habitat types influenced the presence of larvae as well as larval densities ($p < 0.001$). The land-use type affected the presence of *Anopheles* larvae ($p = 0.001$), while vegetation cover influenced larval densities ($p < 0.05$).

Conclusion: The study revealed that the abundance of *Anopheles* breeding habitats and hence *Anopheles* larvae are closely associated with anthropogenic activities. Regulating such activities will lead to a significant reduction in *Anopheles* breeding habitats.

Background

Anopheles mosquitoes are important vectors that transmit diseases, including malaria, lymphatic filariasis among others [1]. The distribution and abundance of adult *Anopheles* mosquitoes are predicated on the presence and productivity of larval breeding habitats [2]. Species of *Anopheles gambiae* complex prefer to breed in shallow water collections that are open to sunlight [3]. Their breeding habitats can be varying sizes of water bodies that are natural or man-made, permanent or temporary, freshwater, or saline [2,4]. *Anopheles funestus*, on the other hand, prefer to breed in shady permanent or semi-permanent water bodies, usually with floating or emergent vegetation such as found in swamps, marshes, and edges of streams [5,6].

The choice of oviposition sites of mosquitoes is influenced by a myriad of environmental factors, which include climatic components such as temperature, rainfall, vegetation, salinity and turbidity of the water, the size of the habitat, and the amount of sunlight [2]. The temperature of larval habitats can influence larval development, pupation rate, and time, as well as larval survivorship [2,7]. Variation in rainfall patterns or seasonal changes can also affect the availability of larval habitats as well as productivity [3].

Vector control is key to the elimination of vector-borne diseases such as malaria [8,9]. Even though the most used vector control methods, long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) have reduced the transmission of malaria in Africa [10,11], these methods have not been successful in malaria eradication because of the emergence and rapid spread of resistance in mosquitoes [12–14]. Also, the use of LLINs and IRS which target indoor biting and indoor resting mosquitoes have driven behavioural changes in the *Anopheles* mosquito from indoor, late-night biting to early biting times when humans might be unprotected outside [15,16]. Nevertheless, larval source management or source control could provide an additional valuable tool for the control of malaria vectors [17]. To assess the feasibility of larval control or larval source management, there is the need to assess the abundance of different types of habitats, measure the productivity in each habitat type [18], and also know how these different habitat types are formed, and how they interact with the society.

The presence and densities of mosquito larvae and consequently, the number of competent adult malaria vectors are regulated by a variety of ecosystem processes interacting at different levels and spatio-temporal scales. These include the presence of water, aquatic plants that protect larvae from predators and serve as detritus that support microbial communities, which, in turn, serves as food for mosquito larvae [19]. Changes in the structure of the ecosystem can have a considerable impact on mosquito populations and species distribution. As such, studies on the ecology of larval habitats should include a landscape context [2,3,20,21]. Landscape features such as topography, land cover and land use influence the formation, distribution, and microclimate conditions of larval habitats which, in turn, influence the distribution of adult *Anopheles* vectors [2,22–25]. Human activities can affect habitat distribution and stability through landscape changes such as deforestation, irrigations, and agricultural practices [2].

There are three main ecological zones in Ghana – the Coastal Savannah in southern Ghana, the Forest in the middle of Ghana, and the Sahel Savannah in northern Ghana. These ecological zones affect the distribution of habitats and importantly, species composition [26]. The coastal savannah and forest zone have a bimodal rainfall pattern, allowing for two peaks of malaria transmission while the Sahel zone has a unimodal rainfall pattern making malaria transmission seasonal.

The ecology of larval mosquitoes has implications for vector control; hence there is the need to understand the productivity and dynamics of larval habitats in the changing environment in efforts to model and predict the abundance of adult mosquitoes and ultimately develop effective control tools or programmes [2,27]. The aim of this study, therefore, was to investigate the ecology of *Anopheles* mosquito larvae in different ecological zones in Ghana. The availability of larval habitats, their productivity, and distribution among different zones in Ghana was studied. The spatio-temporal distribution and species composition of larval malaria vectors were investigated.

Methods

Study sites

The study was conducted in 5 locations in three ecological landscapes of Ghana – the coastal savannah, the forest, and the Sahel savannah zones. Anyakpor (5° 46'51.96 "N 0° 35'12.84 "E) was the site in the coastal savannah area. It is a rural coastal community about 5 km west of Ada Foah, in southern Ghana, and has a dry equatorial climate with temperatures ranging from 23°C – 28°C throughout the year and maximum temperatures reaching 33°C. It has a bimodal rainfall pattern with a long rainy season from April to June and a short rainy season from September to November. It has coastal savannah type vegetation.

Duase (6° 32'3.05 "N 1° 14'42.22 "W) was the site located in the forest zone. It is a rural community close to Konongo. It has a wet semi-equatorial climate characterized by two distinct rainy seasons with a long rainy season from May to July and a short rainy season from September to November. The mean annual temperature and relative humidities are 26°C and 77% respectively.

Kpalsogou (9° 33'45.2 "N 1° 01'54.6 "W), Pagazaa (9° 22'33.34 "N 0° 42'29.67 "W), and Libga (9° 35'32.26 "N 0° 50'48.8 "W) are the selected sites in the sahel savannah region of Northern Ghana. They have a unimodal rainfall pattern from May to November. The mean annual temperature, which is 28°C, appears to be favourable for *Anopheles* larval development, but it can get to a maximum of 42°C.

This study was undertaken during the rainy and dry seasons of 2019.

Larval Habitat Characterization

All larval habitats in each site were classified as natural or man-made. Natural habitats included swamps, streams, and natural ponds whiles man-made habitats included drainage ditches, foot and hoof prints. Land-use type was classified based on the natural vegetation and activities taking place on the land where the larval habitat was found. These include forest for sites with high canopy cover, farmland for cultivated areas; pasture for grazing areas; shrubland for bushy areas with short trees, roads, swamps, and compound or home for places with human settlement. The length and width of each habitat were measured and recorded in metres. The percentage of vegetation cover was also recorded. The vegetation cover was categorized as follows: zero if vegetation were not present in the habitat, ≤ 24% of surface coverage, 25-49%, 50-74%, and 75-100% of surface coverage [28].

Larval Sampling and Densities

Larval sampling was done for all potential breeding sites by the standard dipping method using the WHO 350 ml standard dipper. The size of each habitat was grouped as ≤ 1 m, >1 m – 2 m, >2 m – 5 m, >5 m – 10 m, >10 m – 100 m, and > 100 m and a maximum of 2, 4, 6, 10, 50, and 150 dips were taken respectively (i.e., depending on the size of the habitat) as described by Gouagna and Mereta [28, 36]. For habitats with much smaller sizes such as hoof prints and footprint, a ladle was used to collect the samples. Larvae collected were classified as early instars (L1 and L2) or late instars (L3 and L4). The number of larvae and pupae were recorded, and the larval density was estimated as the ratio of the number of larvae collected per dip [29–32].

Mosquito Species Identification

Anopheline larvae specimens were transported to the insectary of the Department of Medical Microbiology, University of Ghana, where they were bred into adults. The larvae were fed on Tetramin® fish meal and maintained at 27°C ± 2°C. Emerged adult mosquitoes were morphologically identified under a stereomicroscope using the taxonomic keys by Gillies and Coetzee [33]. *Anopheles gambiae* s. l. were further identified to sibling species and molecular forms using rDNA PCR [34] and PCR-RFLP [35] analysis, respectively.

Data Analysis

Descriptive analysis was done to compare the abundance of the various habitat types and larval densities in the different study sites (ecological zones) and seasons. Larval densities were calculated by dividing the total number of larvae collected by the total number of dips taken. The total number of dips for smaller habitats such as footprints and hoof prints were considered to be one dip. A test for normality of the larval density distribution using the Shapiro-Wilk test showed a non-normal distribution. The density of *Anopheles* mosquito larvae was compared among the various breeding habitats and study sites. The Mann-Whitney U and the Kruskal-Wallis test was used to test the associations between continuous and categorical variables. The Chi-square and Fisher's exact tests were used to test the association between two categorical variables. Logistic regression was used to assess the association between the habitat characteristics with categorical data and the presence of *Anopheles* larvae. Nested generalized linear mixed models with sites nested within ecological zones were used to model the effect of habitat characteristics on larval densities. All statistical analyses were conducted in STATA v15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Results

Distribution and abundance of larval habitats in different ecological zones and seasons

A total of 383 breeding habitats made up of 11 different habitat types were encountered and recorded during the study. Most of the habitats were man-made (69.71%, 267/383) and the others (30.29%, 116/383) were natural. The most abundant habitat type was man-made ponds (27.15%, 104/383), which were present throughout the rainy and dry seasons mostly on farmlands. This was followed by natural ponds (12.01%, 46/383), swamps (11.49%, 44/383), dug-out wells (10.97%, 42/383), and concrete wells (9.92%, 38/383). Other habitat types included tyre tracks (7.83%, 30/383), puddles (6.27%, 24/383), and drainage ditches (6.01%, 23/383). The less abundant habitat types were furrows (4.18%, 16/383), hoofprints (2.87%, 11/383), and footprints (1.31%, 5/383) (Table 1).

The distribution of mosquito larval habitat types and their abundance varied among the study sites ($\chi^2 = 498.2658$; $df = 40$; $p = 0.0001$) as well as ecological zones ($\chi^2 = 369.5865$; $df = 20$; $p = 0.0001$) (Additional file 1). In Anyakpor in the coastal savanna zone, only 4 habitats types were encountered; man-made ponds (57%, 97/168), dug-out wells (20.83%, 35/168), concrete wells (20.83%, 35/168), and a natural pond (0.60%, 1/168). In Duase, in the forest, area 8 habitat types were found; natural ponds (29.03%, 18/62), puddles (14.52%, 9/62), tyre tracks (12.90%, 8/62), man-made ponds (11.29%, 7/62), drainage ditches (11.29%, 7/62), dug-out wells (11.29%, 7/62), swamps (6.455, 4/62), and concrete wells (3.23%, 2/62). In Kpalsogu, in the sahel savanna area also, 8 habitat types were encountered. These were swamps (31.50%, 30/96), tyre tracks (21.88%, 21/96), hoofprints (11.46, 11/96), natural ponds (11.46, 11/96), puddles (10.42%, 10/96), furrows (7.29%, 7/96), footprints (4.17%, 4/96), and drainage ditches (2.08%, 2/96). Only 4 habitat types; furrows (28.13%, 9/32), drainage ditches (28.13%, 9/32), natural ponds (21.88%, 7/32), and concrete well (3.13%, 1/32) were encountered in Libga, in the sahel savanna area. In Pagazaa, also in the sahel savanna area, the 6 habitats encountered were natural ponds (36%, 9/25), puddles (20%, 5/25), drainage ditches (20%, 5/25), swamps (16%, 4/25), tyre track (4%, 1/25), and footprints (4%, 1/25). Larval habitats were significantly more abundant in the rainy season (63.45%, 243/383) than in the dry season (36.55%, 140/383) ($\chi^2 = 91.3295$; $df = 10$; $p = 0.001$) (Additional file 1). Dug-out wells were the most abundant habitat type during the dry season while man-made ponds were the most abundant during the rainy season. The presence of larval habitat type was associated with the land use type ($\chi^2 = 587.4192$; $df = 60$; $p = 0.0001$). Larval habitats were mostly found on farmlands (58.75%), followed by pastures (16.19%), and on roads (13.05%). The rest were present in forested areas (4.70%), around homes or compounds (4.18%) by streams and (1.83%), and rivers in swamps (1.31%). Most of the habitats had a vegetation cover of less than 24% ($\chi^2 = 215.1340$; $df = 40$; $p = 0.0001$) (Additional file 1).

Larval habitat types, the presence, and densities of *Anopheles* larvae

The presence of *Anopheles* larvae was dependent on the type of habitat present ($\chi^2 = 41.3651$; $df = 10$; $p < 0.0001$). Even though there was a significant increase in the number of habitats during the rainy season ($\chi^2 = 91.3295$; $df = 10$; $p < 0.0001$) (Additional file 1) compared to the dry season, the proportion of habitats that had the presence of *Anopheles* larvae were not significantly different between the two seasons ($\chi^2 = 0.0051$; $df = 1$; $p = 0.943$) (Additional file 2). In the dry season, 29.29% (41/140) of the habitats had *Anopheles* larvae present while in the rainy season, 29.63 % (72/243) of the habitats were positive for *Anopheles* larvae. In all, dug-out wells were the most inhabited with *Anopheles* larvae during the dry season (39.02%, 16/41) followed by man-made ponds (21.95%, 9/41) whereas during the rainy season man-made ponds were the most inhabited habitat (30.56%, 22/41) followed by swamps (16.67% 12/72) (Table 2).

The type of habitat present was associated with *An.gambiae* s.l larval density ($\chi^2 = 29.593$; $d.f = 10$; $p < 0.001$) (Additional file 3). Even though the mean *An.gambiae* s.l larval density in the rainy season (1.49 larvae/dip) was slightly higher than in the dry season (1.15 larvae/dip), the difference was not significant ($z = -0.232$; $p = 0.8166$). In Anyakpor, the most productive habitat types were dug-out wells during both the dry (1.6 larvae/dip) and rainy (11.28 larvae/dip) seasons (Table 5). The preferred breeding habitat in Kpalsogou were natural ponds during the dry season (0.89 larvae/dip) and swamps (2.57 larvae/dip) during the rainy season. In Libga, drainage ditches were the only habitat type present in the dry season with the mean larval density of 0.3 larvae/dip, while the only productive habitats were furrows in the rainy season (1.83 larvae/dip). There were no productive habitats in Pagazaa during the dry season, but in the rainy season, the most productive habitat type were puddles (1.44 larvae/dip). In Duase, the most productive habitat type was dug-out wells both in the dry (1.47 larvae/dip) and the rainy season (2.05 larvae/dip) (Table 5).

Abundance and distribution of *Anopheles* larvae in the different ecological zones

A total of 7,894 mosquito larvae were collected during this study. Out of this number, 2,152 (27.26%) were Anophelines while 5,742 (72.74%) were culicines. Of the Anopheline species, *An. gambiae* s.l. comprised 2,128 (98.88 %), *An. rufipes* were 16 (0.74 %), whereas *An. pharoensis* was 8 (0.37 %). During the rainy season 1,500 (70.49%) *An. gambiae* s.l were collected while 628 (29.51%) were collected in the dry season.

Anyakpor in the coastal savannah area had the highest abundance of *An. gambiae* s.l larvae (1,286) with 343 (26.67%) occurring in the dry season and 943 (73.33%) in the rainy season (Table 3). During the dry season in Duase, situated in the forest area 30.17% of *An. gambiae* s.l larvae were collected, while 69.83% were collected in the rainy season. Kpalsogou and Libga in the Sahel savannah recorded 39.00% and 35.79% of the *An. gambiae* s.l larvae in the dry season, while the rainy season contributed to 61.00% and 64.21%, respectively. In Pagazaa, also in the Sahel savannah *An. gambiae* s.l larvae were found only in the rainy season (Table 3) and none during the dry season.

The distribution of *An. gambiae* sibling species varied across the ecological zones ($\chi^2 = 45.9887$ $df = 8$; $p = 0.0001$). *Anopheles coluzzii* was the most abundant species (53.44%) in each ecological zone. This was followed by *An. gambiae* s.s. (25.98%), and *An. arabiensis* (6.27%) which were found only in the Sahel savannah; Kpalsogu (19.30%), Libga (17.86%), and Pagazaa (35.71%). *Anopheles melas* were the least abundant species (4.19%) and were present only in Anyakpor in the Coastal savannah area. All the species were more abundant in the rainy season ($\chi^2 = 21.2510$; $d.f. = 2$; $p = 0.0001$) than in the dry season. *Anopheles gambiae* s.s. and *An. coluzzii* were found in all the habitat types encountered in this study. *An. arabiensis* were predominantly found in swamps (52.38 %) and furrows (28.57 %) whereas *An. melas* were found in dug-out wells (55.56 %) and man-made ponds (44.44 %). While *An. rufipes* were found only in Kpalsogu and Libga in the Sahel savannah area, *An. pharoensis* were found in Anyakpor in the coastal savannah area and Libga in the sahel savannah area *Anopheles pharoensis* were found only in man-made ponds (75.00 %) and furrows (25.00 %). *An. rufipes* were found in swamps (56.25 %), footprints (25.00 %), furrows (12.50 %), and puddles (6.25 %).

Habitat characteristics, the occurrence, and densities of *Anopheles* larvae

The type of habitat influenced the presence of *Anopheles* larvae ($\chi^2 = 41.3651$; $d.f. = 10$; $p = 0.0001$) as well as their densities ($p < 0.01$). Significantly higher numbers (84.07% (95/113) of *Anopheles* positive habitats were less than 10 m² in size ($\chi^2 = 11.9217$; $d.f. = 2$; $p = 0.0001$). Land-use type influenced both the presence of *Anopheles* larvae ($\chi^2 = 26.5920$; $d.f. = 6$; $p = 0.0001$) (Additional file 2) and their larval densities ($\chi^2 = 16.117$; $d.f. = 6$; $p = 0.013$) (Additional file 3).

Fifty-five percent (55%) of all larval habitats found around homes or compounds contained *Anopheles* larvae. The odds of finding *Anopheles* larvae in any habitat was twice higher if the vegetation cover was less than 24% (OR = 2.24 [1.02, 4.93]; $p = 0.045$) (Table 6). As the vegetation cover increases, the density of *Anopheles* larvae decreases ($B = -0.016$ [-0.28, 0.003]; $p = 0.015$) (Additional file 4). The present study, again, showed that *Anopheles* larvae have the preference to co-habit with *Culicine* larvae. The likelihood of encountering *Anopheles* larvae in a breeding habitat was over three times higher when culicines are present (OR = 3.13 [1.75, 5.59]; $p < 0.01$) (Table 6).

Discussion

Understanding the ecology of Anopheline larvae in a changing environment is crucial for the development and successful implementation of targeted control measures [36,37] to supplement current adult vector control tools. In this study, the distribution of *Anopheles* breeding habitats in rural communities in the different ecological zones of Ghana has been characterized. These communities were Anyakpor in the coastal savannah area and Duase in the Forest area. Three communities were selected in the Sahel savannah area; Kpalsogu which is an IRS site, Libga where IRS was stopped after 2014, and Pagazaa which has never been under the IRS intervention. The study revealed differences in the abundance and distribution of *Anopheles* breeding habitats in the different ecological zones. Although man-made ponds were the most abundant habitat type, dug-out well the most productive for *Anopheles* mosquito larvae. *Anopheles* larvae also preferred to breed in small habitats while increasing vegetation cover reduced *Anopheles* larval densities.

The common habitat types were man-made ponds, natural ponds, drainage ditches, and swamps. Other habitats such as tyre tracks and puddles were formed usually during the rainy season when rainwater collects on untarred roads [32]. Such habitats are temporal. The formation and abundance of habitat types are greatly influenced by the nature of the topography of the land as well as the land-use [5]. The habitats encountered were mostly associated with anthropogenic activities. This explains why communities that practice irrigation farming, Anyakpor, and Kpalsogou had the highest number of habitats. Because it is a low-lying area with a high-water table, farmers in Anyakpor in coastal Ghana dig wells and ponds to get underground water for irrigation purposes. As a result, wells and man-made ponds were the only breeding habitats encountered in Anyakpor with man-made ponds being the most abundant. In the dry season, when the water table reduces, some of these wells and ponds which serve as breeding habitats dry up.

In contrast to Anyakpor, large dams have been constructed in Kpalsogu and Libga to provide water for both domestic and irrigation purposes during the severe dry season experienced in the Sahel savannah region of Ghana. During this season, most of these dams and other large ponds dry up forming small collections of open sunlit water bodies which are more conducive for *Anopheles* breeding. These findings are similar to previous studies [38–40] which reported that during the dry season, drying water bodies have the most abundant mosquito larvae before the water body becomes completely dried up. On the other hand, during the rainy season, water from the dams and ponds overflow and cause huge swamps and marshes on livestock grazing fields making swamps the most abundant breeding habitats in Kpalsogou. Also, in Libga, and Pagazaa, water from natural ponds is diverted through drainage ditches and furrows onto farmlands, usually rice farms.

Duase and Kpalsogou had the most diverse breeding habitat types, which included eight (8) out of the eleven (11) habitat types encountered. The persistence of breeding habitats during both dry and rainy seasons in the forest and coastal savannah areas account for perennial malaria found within these sites while seasonal variations are observed in the Sahel savanna areas [41,42].

In all, the most abundant habitat type was man-made ponds. Natural ponds are the only habitat type found in all the study sites. Hoofprints were found only in the sahel savannah zone where livestock are left to graze on swampy pastures. Most of the habitats were man-made and found on farmlands. This emphasizes the importance of human activities and, for that matter, land-use in the creation of *Anopheles* breeding habitats and the impact they have on malaria transmission.

The reason why most of the breeding habitats are found on farmland can be attributed to the practice of irrigation. Irrigation provides ideal breeding habitats for *Anopheles* vectors, and this study corroborates with that of Appawu *et al.* [43] who found that irrigated fields generate large numbers of mosquitoes. It is important to note that agrochemicals used on these farms end up polluting the water sources which serve as breeding sites on these farms, thereby leading to the development and spread of insecticide resistance by exposing mosquito larvae to high or sub-lethal doses of agrochemicals [44–47].

Even though 163 of the 383 habitats were inhabited by mosquito larvae, only 29.5% of the had *Anopheles* larvae were present in them. The presence of *Anopheles* larvae also varied significantly based on the habitat type ($p < 0.0001$). The variation in the presence of *Anopheles* larvae may be due to the differences in the physical, chemical, and biological properties as well as the quality of the water present in the various habitats [48]. These properties directly influence the choice of oviposition sites by gravid females and also influence the development and survivorship of larvae [49–52]. *Anopheles* larvae were mostly present in man-made ponds and dug-out wells. This further establishes the role of human activities on the presence and distribution of *Anopheles* vectors [50].

Dug-out wells were the preferred breeding habitats in Anyakpor and Duase, both in the dry and rainy seasons with an increased larval density in the rainy season. In the Sahel savannah area, the preferred breeding habitats for *Anopheles* larvae varied in each site and for each season. With all the sites combined, dug-out wells remained the most productive habitat just as observed by Afrane and co [53]. Dug-out wells were also the most productive habitats both in Anyakpor in the coastal savannah area and Duase in the forest area. In the Sahel savannah area, the most productive habitats were swamps in Kpalsogu, furrows in Libga, and puddles in Pagazaa. It is worthy to note that these habitats that had the highest *Anopheles* larval densities hence major contributors to the *Anopheles* population were man-made or associated with anthropogenic activities which as reported by other studies in Ghana [37,53] and other parts of Africa [31,50,54,55].

Anopheles larvae were always present and also at higher larval densities in habitats less than 10 m² in size. They were not found in habitats that had a perimeter greater than 100 m². More than half of the habitats found around human settlements or homes harboured *Anopheles* larvae and the chances of

finding *Anopheles* larvae in habitats close to human settlements where it was easy to find the next blood meal source increased threefold (OR = 3.06, $p = 0.050$). These findings corroborate with studies from Kenya [56,57] that also suggest that since *An. gambiae* s.l. are closely associated with humans, they will make use of the closest habitat for oviposition when they become gravid [56,57]. Choosing habitats close to human settlements where *An. gambiae* may have taken a blood meal is also an evolutionary strategy to conserve energy [48,58].

This study also revealed that *Anopheles* larvae were predominantly present in breeding habitats with vegetation cover of less than 24%. Similar findings have been observed in Kenya [59–61]. Low vegetation cover allows the habitat to be more exposed to sunlight, a suitable preference for ovipositing mosquitoes [5]. Also, adequate exposure to sunlight warms the water to suitable temperatures as temperature is a key factor that also influences larval development and survival [2,5,7,62]. Inadequate exposure to sunlight, caused by high vegetation cover affects the photosynthetic efficiency of algae biomass which serves as food for mosquito larvae [63]. Evidently, from this study, as the percentage of vegetation cover increased, the density of *Anopheles* larvae decreased ($B = -0.016$, $p = 0.015$). This is in line with studies conducted in Ethiopia [32,39]. As vegetation cover increases, the amount of sunlight reaching the habitat is limited. The study also showed that *Anopheles* larvae preferred to live in sympatry with culicine larvae just as observed by Djamouko-Djonkam and colleagues in Mali [52]. This study, again, showed that the abundance of *Anopheles* larvae increased in the rainy season due to the formation of new breeding habitats [32].

In Anyakpor in the coastal zone, the predominant species was *An. coluzzii* (60.82 %) similar to what was reported by Kudom [64] and Fossog *et al.*, [65] whose model shows *An. coluzzii* to dominate the coastal line of Africa. They were mostly found in man-made ponds dug-out wells and concrete wells. *Anopheles gambiae* s.s contributed to 20.62% of the species in Anyakpor. Compared to *An. gambiae* s.s., both inland and coastal *An. coluzzii* are known to have a higher tolerance to salinity [65,66]. *Anopheles melas* in Anyakpor were found only in dug-out wells and man-made ponds because these habitats are fed by salty underground water which *An. melas* prefer to breed in, unlike concrete wells which were fed by rainwater. *Anopheles coluzzii* and *An. gambiae* s.s were the only species found in Duase, in the forest zone, with *An. coluzzii* being the dominant species which is contrary to the findings of other studies [41,67,68]. This could be as a result of the deforestation which is caused by rapid urbanization. Rapid deforestation affects climatic conditions, and this might be causing Duase to become drier, thereby making *An. coluzzii* to thrive there. *Anopheles coluzzii* were found in drainage ditches, natural ponds, and tyre tracks. *Anopheles gambiae* s.s were found in dug-out wells, puddles, tyre tracks, and drainage ditches. *Anopheles coluzzii* was also the dominant species in Kpalsogou followed by *An. gambiae* s.s. and *An. arabiensis*. On the other hand, *An. gambiae* s.s. was the dominant species in Libga. In Pagazaa, *An. coluzzii* and *An. arabiensis* were co-dominant. *Anopheles arabiensis* were found only in the Sahel savanna area because of its dry sub arid environments [69]. *Anopheles arabiensis* was predominantly found in swamps and furrows. Furrows were mostly present on rice farms. *Anopheles arabiensis* prefer to be zoophilic even though they can also be anthropophilic [70] and this can account for the reason they were mostly found in swamps since swamps in the Sahel savannah areas mostly serve for grazing cattle. As shown by this study, *An. coluzzii* and *An. gambiae* s.s are known to live in sympatry in most parts of Ghana [64]. However, *An. coluzzii* predominates in the Coastal and Sahel savannah regions of Ghana [67,68,71]. The dominance of *An. coluzzii* in the coastal regions of Ghana has been attributed to permanent habitats created on irrigation farms [67,68]. *Anopheles coluzzii* is usually seen to be breeding in large permanent habitats, but in this study, they were also found in small temporary habitats such as hoofprints. Studies done by Edillo *et al.*, (2006) showed that *An. coluzzii* preferred different breeding habitat types to *An. gambiae* s.s. and *An. arabiensis* but this study showed otherwise. *Anopheles gambiae* s.s and *An. coluzzii* were found in all 11 habitat types.

This study involved field surveys carried out under natural conditions and external factors. These factors could have influenced the presence and development of mosquito larvae in the various habitats. Studies on the ecology of *Anopheles* mosquito larvae are methodologically challenging as such has several limitations. The standard dipping technique adapted for this study was not suitable for sampling in small habitats especially footprints and hoofprints. Consequently, the larval densities in these habitats may have been overestimated in this study. In this study, larval density was used to express the productivity of a larval habitat [20,30] instead of the number of pupae or adult mosquitoes that emerged from the habitat [56,72].

This study provides a baseline insight into the development of integrated larval source management suitable for the larval habitats in the various study sites. This study revealed that irrigated farms contribute to higher populations of malaria mosquitoes as this system of agriculture creates a lot of habitats suitable for *Anopheles* mosquito larvae. This made communities that practice irrigation farming have a higher abundance of *Anopheles* larvae. This study, again, revealed that human activities contribute greatly to the presence and abundance of *Anopheles* mosquito larvae. This implies that changes in agricultural methods and environmental management would be extremely appropriate in controlling malaria transmission. Considering that *An. gambiae* s.l prefers to breed in small habitats, habitat modification would be a suitable method of larval source reduction. Other integrated larval source management approaches such as water and environmental management and biological control methods are feasible. In communities that rely on the same water collections for domestic purposes, and in situations where the water collections cannot be drained, microbial bio-larvicides may be used.

Conclusion

This study showed that the presence and availability of *Anopheles* breeding habitats varied among the study site, hence, ecological zones. The abundance of breeding habitats was influenced by rainfall as well as the topography of the land, as more habitats are created during the rainy season and this, in turn, increases the abundance of *Anopheles* mosquitoes. Even though man-made ponds were the most abundant breeding habitat types, dug-out wells were the most productive. This established the role of human activities in creating suitable breeding habitats for *An. gambiae* s.l. *Anopheles gambiae* s.l larvae preferred to breed in small habitats that also have vegetation cover less than 24%. *Anopheles* larvae also preferred to live in sympatry with culicine larvae. *Anopheles coluzzii* was the predominant species in all the study sites. They also were usually in sympatry with *An. gambiae* s.s.

Declarations

Ethics approval and consent to participate

Ethical approval for this study with protocol identification number CHS-Et/M2 -5.5/2019-2020 was obtained from the Ethics and Protocol Review Committee of the College of Health Sciences, University of Ghana.

Consent for Publication

Not applicable

Availability of data and materials

All datasets generated and/or analysed during the current study are available on request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

IAH, SKA, AOF, and YAA conceived and designed the study. IAH and YAA were responsible for designing and coordinating the entomological surveys. IAH was responsible for data collection. YAA, SKA, and AOF supervised the data collection. IAH and BAM were responsible for the data analysis and drafted the first manuscript. All authors read and approved the final copy of the manuscript.

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Tables

Table 1: Distribution and Abundance of Larval Habitat

	Concrete well	Dug-out well	Natural pond	Man-made pond	Drainage ditch	Tyre track	Footprint	Hoofprint	Swamp	Furrow
Dry season										
Anyakpor	5 (62.50)	27 (90.00)	0	22 (95.65)	0	0	0	0	0	0
Duase	2 (25.00)	3 (10.00)	9 (31.03)	1 (4.35)	4 (22.22)	0	0	0	2 (10.53)	0
Kpalsogou	0	0	9 (31.03)	0	2 (11.11)	0	1 (100)	1 (100)	16 (84.21)	1 (100)
Libga	1 (12.50)	0	5 (17.24)	0	9 (50.00)	0	0	0	0	5 (100)
Pagazaa	0	0	6 (20.69)	0	3 (16.67)	0	0	0	1 (5.26)	0
Total	8 (100)	30 (100)	29 (100)	23(100)	18 (100)	0	1 (100)	1 (100)	19 (100)	6 (100)
Rainy season										
Anyakpor	30 (100)	8 (66.67)	1 (5.88)	75 (92.59)	0	0	0	0	0	0
Duase	0	4 (33.33)	9 (52.94)	6 (7.41)	3 (60)	8 (26.67)	0	0	2 (8)	0
Kpalsogou	0	0	2 (11.76)	0	0	21 (70)	3 (75)	10 (100)	14 (56)	6 (60)
Libga	0	0	2 (11.76)	0	0	0	0	0	6 (24)	4 (40)
Pagazaa	0	0	3 (17.65)	0	2 (40)	1 (3.33)	1 (25)	0	3 (12)	0
Total	30 (100)	12 (100)	17 (100)	81 (100)	5 (100)	30 (100)	4 (100)	10 (100)	25 (100)	10 (100)

Numbers in parenthesis are percentages

Table 2: Larval habitat types and the presence of larvae during the dry and rainy seasons

Habitat type	Total No. (%) of breeding habitats		No. (%) of habitats with mosquito larvae present		No. (%) of habitats with <i>Anopheles</i> spp. present	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
Concrete well	8 (5.71)	30 (12.35)	1 (1.89)	10 (9.09)	0 (0.00)	9 (12.50)
Dug-out well	30 (21.43)	12 (4.94)	18 (33.96)	8 (7.27)	16 (39.02)	6 (8.33)
Natural pond	29 (20.71)	17 (7.00)	7 (13.21)	5 (4.55)	3 (7.32)	3 (4.17)
Man-made pond	23 (16.43)	81 (33.33)	10 (18.87)	47 (42.73)	9 (21.95)	22 (30.56)
Drainage ditch	18 (12.86)	5 (2.06)	7 (13.21)	1 (0.91)	4 (9.76)	1 (1.39)
Puddle	5 (3.57)	19 (7.82)	2 (3.77)	4 (3.64)	2 (4.88)	4 (5.56)
Tyre track	0 (0.00)	30 (12.35)	0 (0.00)	5 (4.55)	0 (0.00)	2 (2.78)
Footprint	1 (0.71)	4 (1.65)	0 (0.00)	3 (2.73)	0 (0.00)	3 (4.17)
Hoofprint	1 (0.71)	10 (4.12)	0 (0.00)	4 (3.64)	0 (0.00)	2 (2.78)
Swamp	19 (13.57)	25 (10.29)	4 (7.55)	15 (13.64)	4 (9.76)	12 (16.67)
Furrow	6 (4.29)	10 (4.12)	4 (7.55)	8 (7.27)	3 (7.32)	8 (11.11)
Total	140 (100)	243 (100)	53 (100)	110 (100)	41 (100)	72 (100)

Numbers in parenthesis are percentages

Table 3: Anopheles larval density in the dry and rainy seasons

Habitat type	Larval density (larvae/dip)									
	Anyakpor		Duase		Kpalsogou		Libga		Pagazaa	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
Concrete well	0	1.23	0	0	0	0	0	0	0	0
Dug-out well	1.59	11.28	1.47	2.05	0	0	0	0	0	0
Natural pond	0	0	0.09	0.03	0.89	0	0	0	0	0.15
Man-made pond	0.56	0.84	1.20	0.97	0	0	0	0	0	0
Puddle	0	0	0.44	0	0	0.25	0	0	0	1.44
Drainage ditch	0	0	0.55	0.25	0	0	0.30	0	0	0
Tyre track	0	0	0	0.9	0	0	0	0	0	0
Furrow	0	0	0	0	0	2.35	0.21	1.83	0	0
Hoofprint	0	0	0	0	0	0.46	0	0	0	0
Footprint	0	0	0	0	0	2.00	0	0	0	0.2
Swamp	0	0	0	0	0.27	2.57	0	1.13	0	0.15

Table 4: Distribution of larval Anopheles species in the study sites

Anopheles larvae	Sites N (%)					Total
	Anyakpor	Duase	Kpalsogou	Libga	Pagazaa	
Anopheles gambiae s.l						
An. gambiae s.s	265 (20.62)	75 (42.11)	132 (31.58)	75 (39.29)	12 (21.43)	559 (25.98)
An. coluzzii	782 (60.82)	104 (57.89)	176 (42.11)	68 (35.71)	20 (35.71)	1150 (53.44)
An. arabiensis	0	0	81 (19.30)	34 (17.86)	20 (35.71)	135 (6.27)
An. melas	119 (9.28)	0	0	0	0	119 (5.53)
Unidentified An. gambiae species	119 (9.28)	0	29 (7.02)	13 (7.14)	4 (7.14)	165 (7.67)
Other Anophelines						
An. pharoensis	6 (0.46)	0	0	2 (1.03)	0	8 (0.37)
An. rufipees	0	0	13 (3.02)	3 (1.54)	0	16 (0.74)
Total	1,292 (100)	179 (100)	431(100)	195(100)	55 (100)	2152 (100)

Table 5: Abundance of larval *An. gambiae* s.l larvae during the dry and rainy season

Study Site	No. (%) of <i>An. gambiae</i> s.l		Total (%)
	Dry Season	Rainy Season	
Anyakpor	343 (54.62)	943 (62.87)	1286 (60.43)
Duase	54 (8.60)	125 (8.33)	179 (8.41)
Kpalsogu	163 (25.96)	255 (17.00)	418 (19.64)
Libga	68 (10.83)	122 (8.13)	190 (8.93)
Pagazaa	0 (0.00)	55 (100)	55 (2.59)
Total	628 (100)	1500 (100)	2128 (100)

Table 6: Logistic regression table showing habitat characteristics that influence the presence of *Anopheles* larvae

Characteristic	Categories	<i>Anopheles</i> present	<i>Anopheles</i> absent	Adjusted OR (CI)	p-value
Habitat type	Concrete well	9/38 (23.68)	29/38 (76.32)	1	
	Dug-out well	22/42 (52.38)	20/42 (47.62)	2.59 (0.87, 7.54)	0.107
	Natural pond	6/46 (13.04)	40/46 (86.96)	0.27 (0.01, 9.27)	0.468
	Man-made pond	31/104 (29.81)	73/104 (70.19)	0.83 (0.30, 2.28)	0.714
	Drainage ditch	5/23 (21.74)	18/23 (78.26)	2.26 (0.23, 21.98)	0.483
	Puddle	6/24 (25.00)	18/24 (75.00)	0.51 (0.02, 17.20)	0.710
	Tyre track	2/30 (6.67)	28/30 (93.33)	6.43 (0.24, 173.44)	0.268
	Footprint	3/5 (60.00)	2/5 (40.00)	24 (1.57, 386.77)	0.023
	Hoof print	2/11 (18.18)	9/11 (81.82)	2.26 (0.14, 35.30)	0.562
	Swamp	16/44 (36.36)	28/44 (63.64)	1.33 (0.04, 43.59)	0.870
	Furrow	5/16 (31.25)	11/16 (68.75)	8.17 (0.64, 104.94)	0.107
Nature of habitat	Natural	33/116 (28.45)	83/116 (71.55)	1	
	Man-made	80/267 (29.96)	187/267 (70.04)	0.15 (0.01, 2.74)	0.203
Habitat size categorical	< 10 m	95/295 (32.20)	200/295 (67.80)	1	
	10 – 100 m	18/62 (29.03)	44/62 (70.97)	1.73 (0.74, 4.01)	0.203
	> 100 m	0	26/26 (100)	1	
Vegetation cover categorical	None	21/92 (22.83)	71/92 (77.17)	1	
	< 24 %	38/98 (38.78)	60/98 (61.22)	2.24 (1.02, 4.93)	0.045
	25 – 49 %	14/44 (31.82)	30/44 (68.18)	2.62 (0.50, 13.74)	0.253
	50 – 74 %	16/47 (34.04)	31/47 (65.96)	3.06 (0.20, 46.04)	0.419
	75 – 100 %	74/95 (77.89)	21/95 (22.11)	2.61 (0.07, 101.11)	0.607
Land-use Type	Farmland	72/225 (32.00)	153/225 (68.00)	1	
	Pasture	23/62 (37.10)	39/62 (62.90)	0.82 (0.29, 2.33)	0.716
	River/stream	0/7	7/7 (100)	1	
	Swamp	0/5	5/5 (100)	1	
	Road	3/50 (6.00)	47/50 (94.00)	0.10 (0.01, 0.95)	0.045
	Compound/home	10/18 (55.56)	8/18 (44.44)	3.06 (1.00, 9.36)	0.050
	Forest	5/16 (31.25)	11/16 (68.75)	1.05 (0.24, 4.55)	0.943
Season	Dry	41/140 (29.29)	99/140 (70.71)		
	Rainy	72/243 (29.63)	171/243 (70.37)	1.55 (0.83, 2.92)	0.171
Presence of Culicines	Absent	59/279 (21.15)	220/279 (78.85)	1	
	Present	54/168 (32.14)	114/168 (67.86)	3.61 (2.00, 6.53)	0.0001

Figures

Figure 1

Map of study area Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Pictures of some habitat types found during the study period. A: Dugout well; B: Concrete well; C: Hoofprints; D: Man-made pond; E: Swamp; F: Furrow; G: Footprint; H: Natural pond; I: Drainage ditch; J: Tyre track; K: Puddle

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