ORIGINAL ARTICLE

Characterisation of breeding sites of anopheline mosquitoes in District Bannu, KPK, Pakistan

Sadiq Ullah Khan, Rahmat Ali Khan

Abstract

Objective: To highlight the role of habitat evaluation in reducing the potential transmission risk of malaria.

Methods: This study was conducted from January to June, 2015, in District Bannu in Khyber Pakhtunkhwa, Pakistan, where 64 larval habitats were characterised in 10 villages of the district. The larvae habitat features, like its permanent or temporary nature, artificial or natural, basic type, substrate type and vegetation, anopheline and culicine larval presence and density, were noted. ArcGIS 9.2 was used to map the mosquitoe breeding sites. Data was analysed related to the effect of temperature, rainfall and relative humidity on larval occurrence and density.

Results: Of the 64 breeding habitats characterised, 26(40.6%) were temporary, while the remaining 38(59.4%) were permanent. Anopheline larvae were found in different types of habitats and occurred in man-made and temporary habitats with high population density. The marshlands (rice fields, sugarcane and open drains) were positive for anopheline larvae. The climatic factors like rain and humidity positively affected the larval density. The larval density was high in March and April at temperatures ranging from 16.1Co-23.45oC.

Conclusion: Targeting the man-made and temporary larval habitats could results in the effective anopheline mosquitoes larvae control.

Keywords: Larvae breeding sites, Larval control, Global Positioning System, Malaria, Bannu, Anopheline.
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Introduction

Mosquitoes are responsible for a wide range of vector-borne diseases, including malaria.[1] It is transmitted by female mosquitoes of Genus Anopheles.[4] It is endemic in tropical and sub-tropical regions where 300 million people are infected with a death of one million each year.[2] Malaria continues to be a major health problem and it is going out of control because of parasite resistance to drugs, insecticide resistance of vector and unplanned land use changes.[3]

The environmental changes including land use and climatic variability have great impact on malaria transmission.[8] Increased deforestation, agriculture expansion, rapid growth of population and urbanisation may increase the proliferation of malaria vector breeding sites.[9] The effect of climatic variability on malaria transmission has been detected in the eastern and western highlands of Africa.[4,5] Agriculture activities often need moist, disturbed environments that consist of both larval and adult mosquitoes.[9] Urban farming is sustained by water storage and irrigation, thereby increasing the availability of mosquito-breeding sites.[6] Many Anopheles species have been reported from the habitats in agricultural area in the highlands.[7]

Small dams and hydropower dams may have the impact on malaria transmission due to presence of larval breeding site.[8]

Chemical insecticides are largely used for the control of malaria and many have been recommended for the public health use.[9] These chemical insecticides are largely used for indoor spraying and mosquito nets.[10] In Pakistan 24 species of the genus anopheles have been reported.[11] The anopheles stephensi and anopheles culicifacies are the confirmed vector of malaria in Pakistan.[12] The development of resistance against chemical insecticides is a serious threat to malaria control programmes.[13] Therefore there is need to develop alternative vector control tools and should be taken into consideration to minimise the human vector contact and hence malaria transmission intensity. To control adult mosquitoes is difficult, time-consuming and labour-requiring task. Alternatively larval habitats management is effective in mosquito control.[14] The environmental management is gaining importance for larval and malaria control.[15] It involves techniques like draining wetlands, fillings small ponds or water holding depressions and land-levelling to reduce mosquito-breeding sites.[16]

To reduce the malaria transmission anopheline larval

University of Science and Technology, Bannu, Pakistan.
Correspondence: Rahmat Ali Khan. Email: rahmatgul_81@yahoo.com

habitat characterisation requires the detailed knowledge of anopheline ecology.[17] In District Bannu the landscape consists of many rivers that provide the breeding sites for the malaria vector. Seasonal flooding and rains create large area of water for the breeding sites of mosquitoes.[18] There is substantial variation in the incidence of malaria across different villages of Bannu. The national strategy to control malaria in Pakistan is the use of insecticide residual spray and bed-nets distribution.[19] However, the larval control of anopheles will show the efficacy and the residual effect.

The understanding of spatial distribution and temporal occurrence of the malaria vector is easily done by mapping of larval habitats in the area which provides a data regarding malaria transmission for malaria-control programmes. Geographic Information System (GIS) is a useful tool for analysis of health-related data. It is continuously used for analysing health-related data in developed and developing countries. By the use of GIS, management, allocation of resources, and preparation of the needs for control of disease in high-risk regions could be easily done. This tool also allows preparing the revised data as soon as new data is available.[20]

Using a Global Positioning System (GPS) device to map the larval breeding sites of mosquitoes, the current study was done to mention specific features of larval breeding sites and that they were related with the occurrence of anopheline larvae.[21,22] The study was planned to highlight that habitat evaluation should be considered as part of efforts to reduce the potential transmission risk of malaria.

Materials and Methods

This study was conducted from January to June, 2015, in District Bannu in Khyber Pakhtunkhwa, Pakistan, where 64 larval habitats were characterised in 10 villages of the district. Bannu is the important road link between Kark and Dera Ismail khan. The estimated population is 6 77,346 inhabitants over an area of 1227 square km with a density of 552 inhabitants per km^2. Two rainy seasons occur; one during March and the other during July-August (summer monsoon). The two rivers, Kurrum and Gambila (Tochi), that originate from the hills of Waziristan are the main sources of irrigation water in Bannu. These rivers may possibly provide favourable mosquito-breeding sites as they traverses the areas. The valley of Bannu spreads to the frontier hills in the form of oval shape measuring 100 km from north to south and 60km from east to west. Being an agriculture area, Bannu is fed by extensive network of irrigation system as wide land area of Bannu is cultivated. Due to increased urbanisation and unplanned sewerage facilities, the stagnant water may be present to work as breeding sites for anopheline mosquitoes. The focus of this study was the ten randomly selected villages of Bannu, namely Bannutownship, Surani, Mamashkel, Shamshikhel, Taji Kula, Mandan, Kakki, Baistkhel, Akhund Kheland Nurur.

The survey of malaria vector breeding sites where water stagnates was performed. The breeding sites were identified and classified according to their origin. For example, habitats present along the river bank were named as river fringe because they were formed when the level of water dropped in the river or formed along the bank of river due to water seepage. Similarly, others were named as rain-pools, drainage-pool, artificial watering points like stagnant water in parks, man-made ponds, irrigation channel margins, and drainage channels. Similarly, pits for brick manufacturing and plastering, streamed pools, natural wetlands and animal hoof prints were also sampled.

The habitats were also characterised according to the vegetation present in it. Habitats were named for vegetation by different categories like agriculture crops (grass, daffodils, date palm, rice, jute) algae and tress canopy. The breeding site without any vegetation was categorised with no vegetation. The vegetation was also noted as emergent, submerged and floating type. The canopy of vegetation for each breeding site was also recorded visually in percentage. Canopy cover was defined as the amount of vegetation covering the water surface and was grouped as low (10-40%), moderate (40%), high (upto 60%) and very high (90%).

The sampling sites were also identified according to its stability as temporary and permanent. Habitats holding water after rain for short period upto approximately for two weeks were categorised as temporary larval habitats. Those holding water after the rainy season has been ended or fed by other sources for long period up to one year were grouped as permanent habitats. The temporary habitats changed according to the availability of water while the permanent habitats remain at the same location.

The larval density was determined in all defined larval habitats during the entire study period. The sampling was done according to standard dipping method.[23] The 350ml mosquito dipper was used. Each village was visited twice-a-month for larval density determination. Only 30 habitats were surveyed for six months. The remaining were surveyed only once. The number of dips depended on the perimeter of the habitats. For example from a habitats of $\leq$10m only 5 dips, from 10-50m only 10 dips and greater than 50 only 15 dips were taken. The larval density was then expressed as larvae per dip (total number of larvae/no of dips). The anopheline larvae were identified by resting and feeding behaviour. Also many larval stages were identified under microscope.[24]

The climatic data was obtained by requesting from the meteorology department of Khyber Pakhtunkhwa. The data regarding rain, humidity and temperature from January to June 2015 was obtained. This data was meant for malaria risk analysis in the study localities. The impact of climatic condition on larval density was also determined.

The breeding sites of 10 localities were mapped with the help of hand-held GPS device (Germin Oregon 550). The Google map was used as reference to identify the boundary of study area. The coordinates of larval breeding sites in 10 localities of each were recorded by GPS. The mapping points noted was latitude, longitude and elevation for each breeding site. Also the distance of nearest community to breeding site was pointed out. The coordinates were tabulated in an Excel file. Using the ArcMAP 10 GIS software the coordinates were integrated to develop a map of breeding sites of the study area.

Results

Of the 64 breeding habitats characterised, 26(40.6%) were temporary in nature, while the remaining 38(59.4%) were permanent (Figure-1). Each village showed different types of habitats based on artificial and natural (main class), type, substrate present and the presence of anopheles (Table-1).

Occurrence of artificial breeding sites was high compared to natural breeding sites of malaria vector, giving an

Table-1: Input variable for the larval habitats characterisation.

Main class: Artificial or Natural
Type (sub-class): Marshlands, Rain pools, Drainage pools, Man-made ponds, Artificial watering points, Irrigation channels margins, Brick manufacturing ponds and River fringes.
Nature of occurrence: Permanent or Temporary
Vegetation: Algae, Grass, Trees, Rice, Sesbania, Acacia plants, Daffodils, Date palm (submerged, emerged, floating)
Substrate: Silt, Gravel, clay, Artificial substrate (plastic pieces),
Competitors: Water scorpion, back swimmers, water bugs, tadpoles
Predators: Water beetle, dragon fly nymphs, dams fly
Anopheline: Presence or Negative

Table-2: Characterisation of mosquitoes larval breeding habitats in the study area.

Main class	Sub-class	No.	Substrate	Temporary	Permanent	Canopy cover
Artificial	Man-made watering point(Parks+ tube well tanks)	6	Concrete,	4	2	No vegetation
Artificial	Irrigation channels margins	7	muddy	3	4	Floating grass
Artificial	Man-made ponds	6	muddy	3	3	Floating algae
Artificial	Brick manufacturing ponds	8	Silt	0	8	Submerged vegetation
Artificial	Drainage pools	10	Artificial substrate	4	6	Emerged grass
Natural	Rain pool	6	Artificial substrate+ gravel	6	0	Floating algae
Natural	Marshlands	15	Muddy	3	12	Rice plants
Natural	River fringe	6	Gravel	3	3	Daffodils
Total		64	--	26	38	--

Table-3: Occurrence of anopheline larvae in different types of larval habitats.

No.	Habitats types	No. of samples N=64	Anopheline positive samples n	Culicine positive	Combined habitats
1	Artificial watering points(fountain in parks, stagnant water near tube well,)	6	3	2	1
2	Irrigation channels margins	7	4	3	0
3	Man-made ponds	6	2	2	2
4	Brick manufacturing ponds	8	2	4	2
5	Drainage pools	10	3	5	2
6	Rain pool	6	6	0	0
7	Marshlands(Rice paddies, open drains, other farm lands)	15	8	4	3
8	River fringe	6	3	1	2
	Total	64	37	15	12

Table-4: Month wise Average temperature, Relative humidity (RH), Average rain fall and average larval density (Ld) in study area.

Month	Av. Temperature(Co)	Rainfall(mm)	Av. RH(%)	Av.AnophelineLd	No. of Cases detected
January	10.35	25	62	27	240
February	13.65	42.4	65	73	207
March	16.1	85.4	67.5	152	325
April	23.25	16.3	52.5	341	462
May	29.55	.5	33.5	422	435
Jun	32.1	20	22.5	236	322

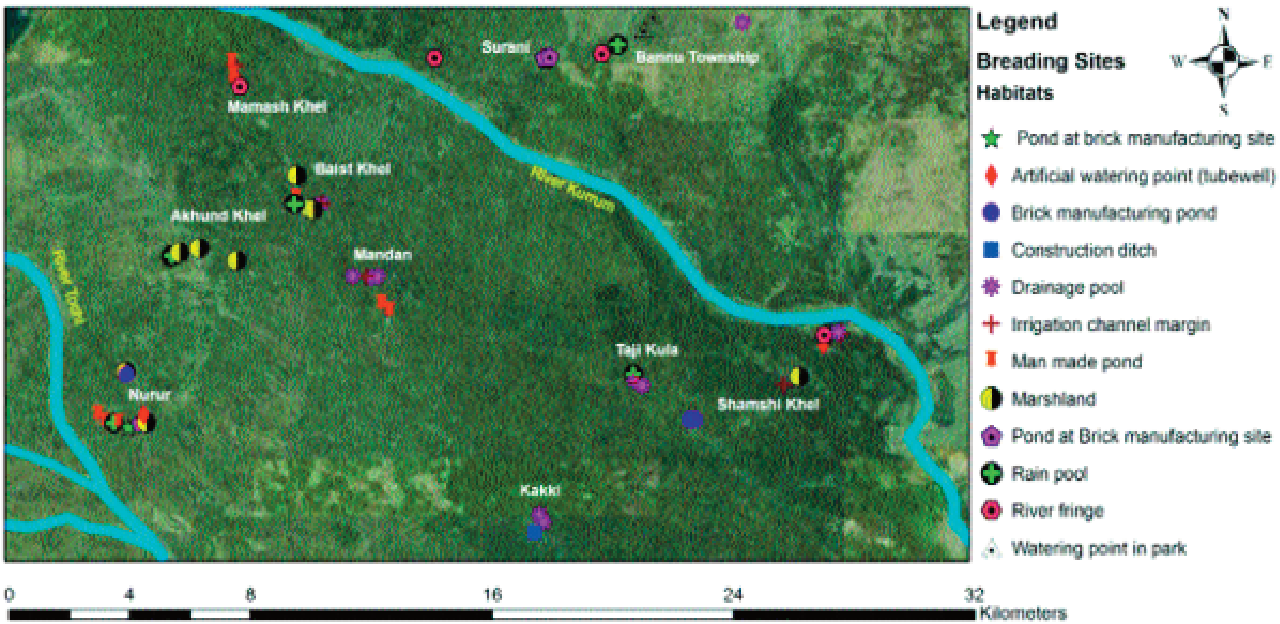

Figure-1: Distribution of larval habitats in 10 villages of District Bannu.

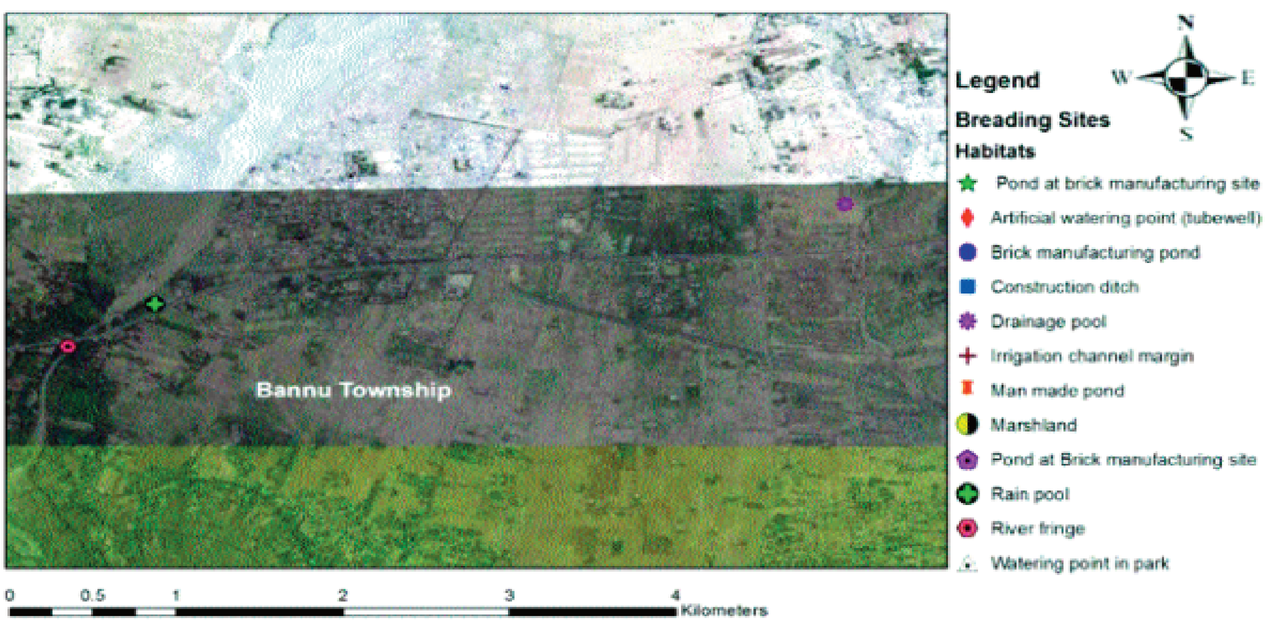

Figure-2: Distribution of larval habitats in the Bannu Township.

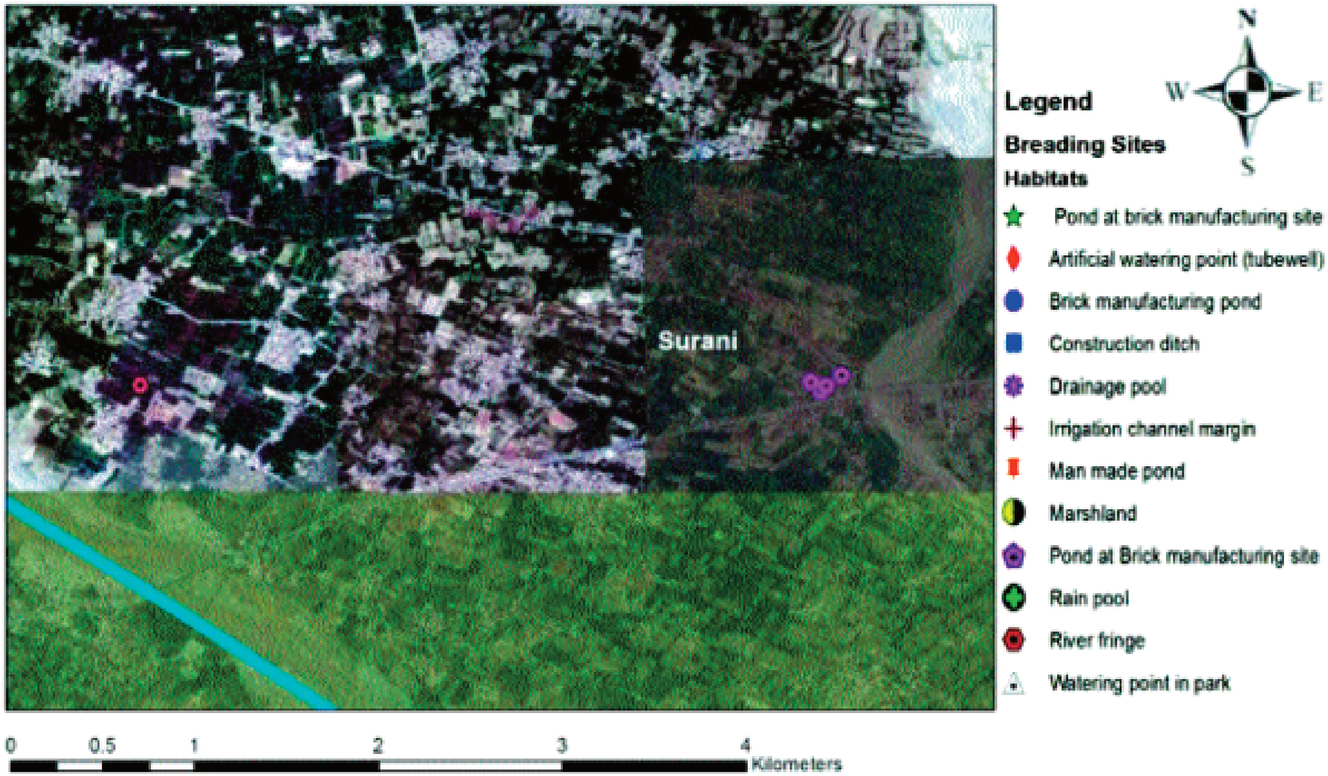

Figure-3: Distribution of larval habitats in Village Surani.

Figure-4: Distribution of larval habitats in Village Mamashkhel.

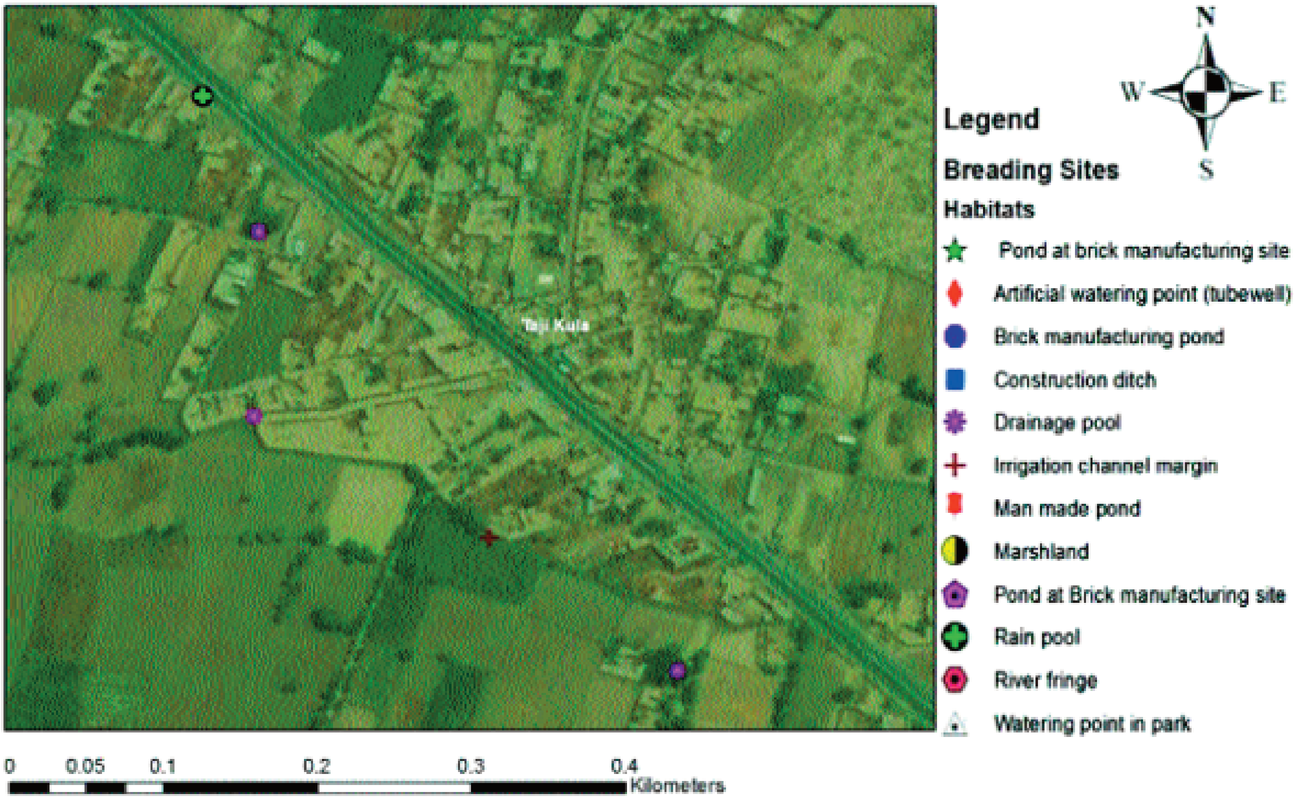

Figure-5: Distribution of larval habitats in Village Taji Kula.

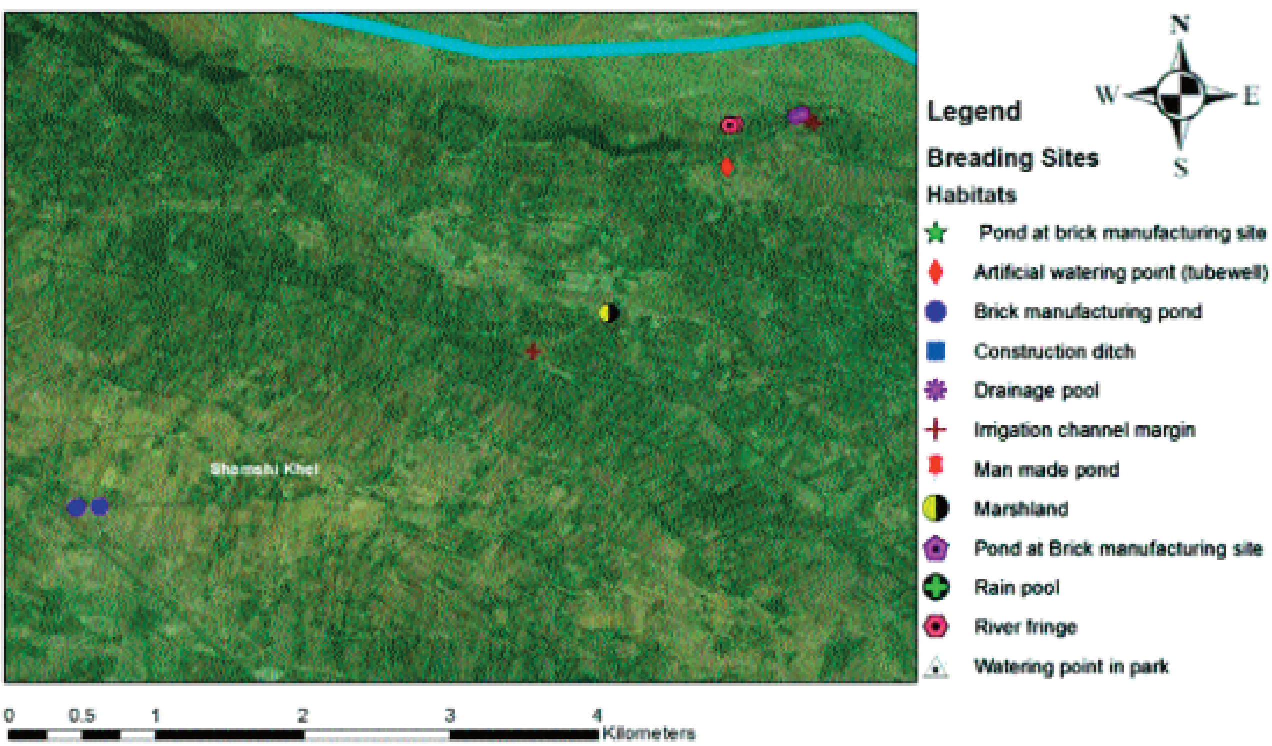

Figure-6: Distribution of larval habitats in Village Shamshikhel.

Figure-7: Distribution of larval habitats in Village Mandan.

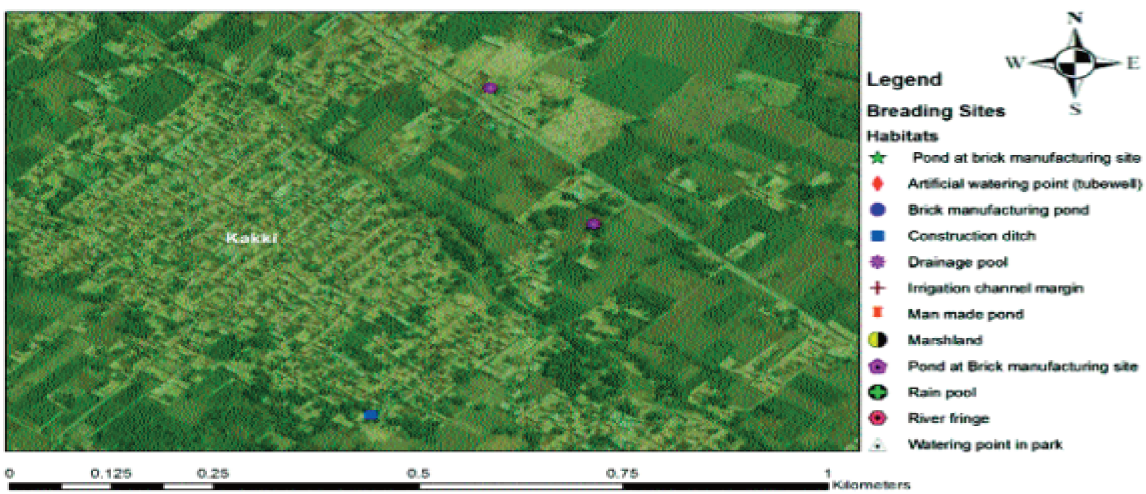

Figure-8: Distribution of larval habitats in Village Kakki.

Figure-9: Distribution of larval habitats in Village Baistkhel.

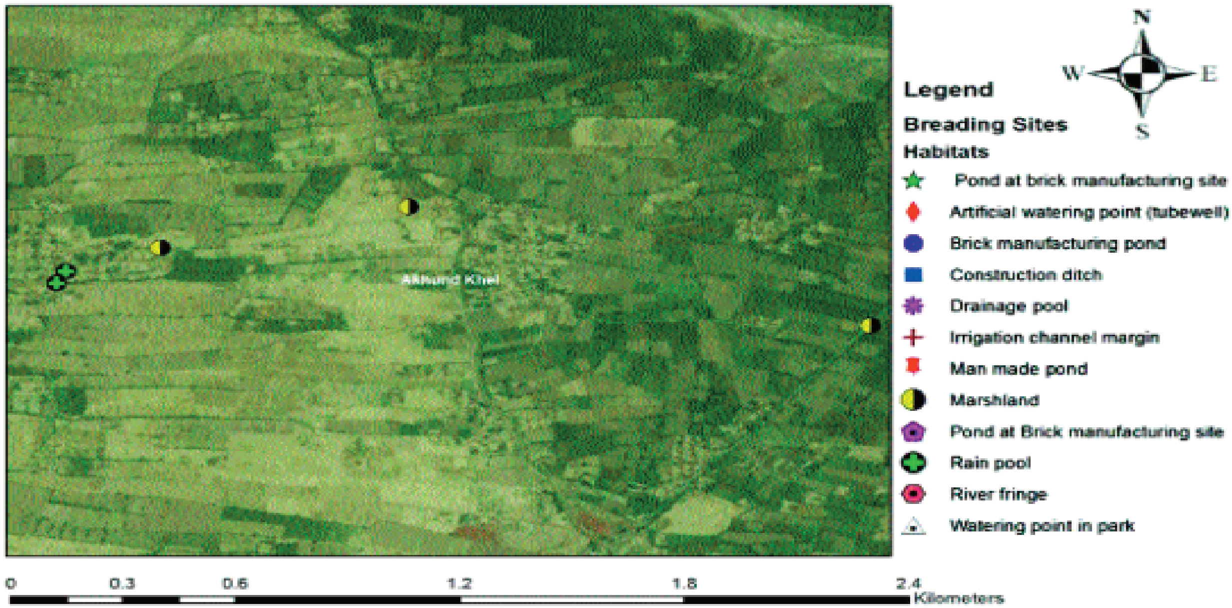

Figure-10: Distribution of larval habitats in Village Akhundkhel.

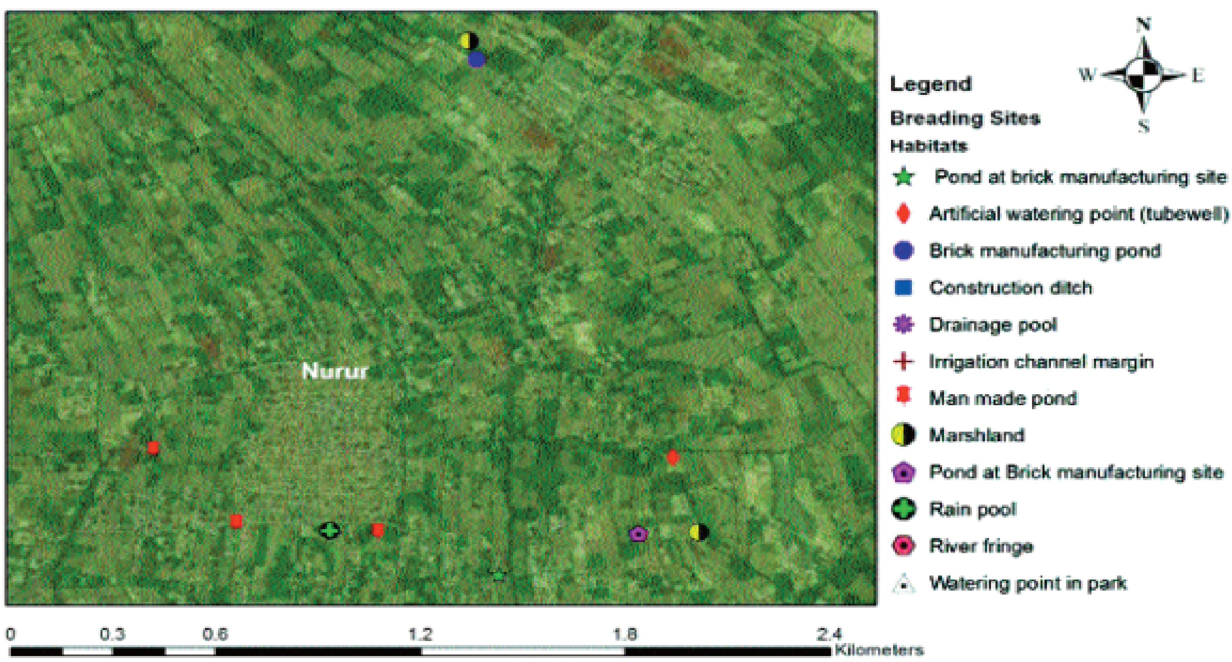

Figure-11: Distribution of larval habitats in Village Nurur.

impression that man-made activities are impacting more on malaria incidence. It also showed that drainage pools and brick manufacturing ponds were greater in number compared to other sub-classes of breeding sites.

The villages (Figure-2-11) with cultivated lands had the highest number of larval breeding sites. These larval habitats includes the river fringes, rain pools, drainage pools, irrigation channel margins, man-made ponds, brick-manufacturing ponds, marshlands and artificial watering points. The studied villages consisted of different types of larval habitats, the water source of them may be river, irrigation streams, drainage system, rain and man-made activities (construction site ditches, tube wells, farming/agriculture). The marshlands were present in high number compared to other types of habitats.

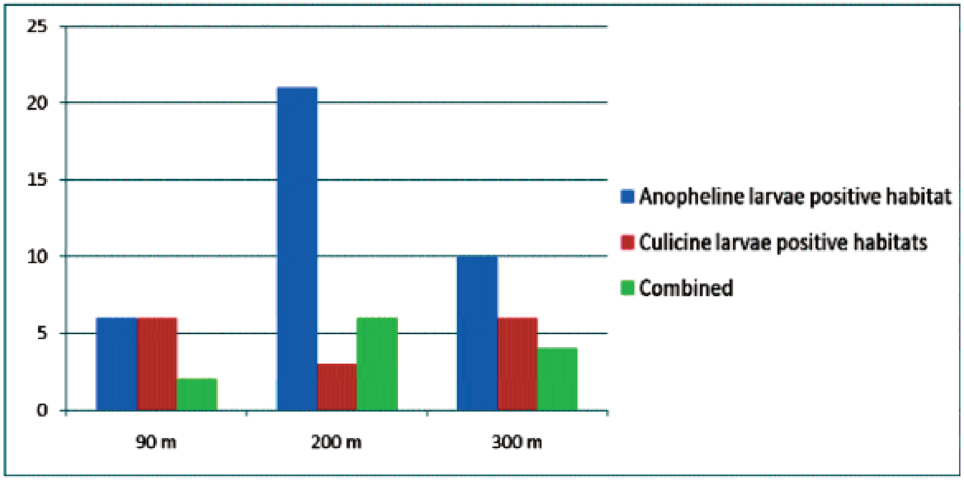

Figure-12: Occurrence of anopheline and culicine larval habitats with different from the nearby community.

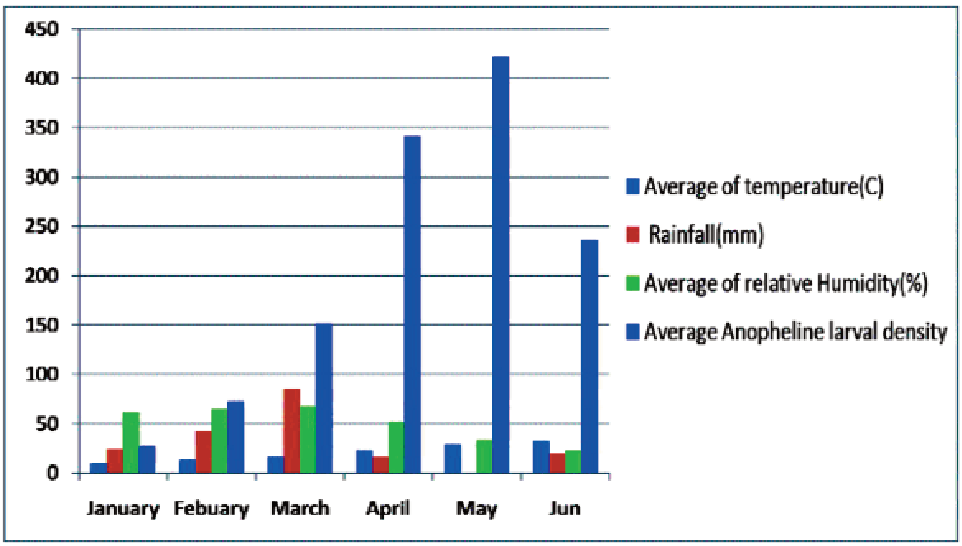

Figure-13: Larval density in relation to climatic factors in district Bannu.

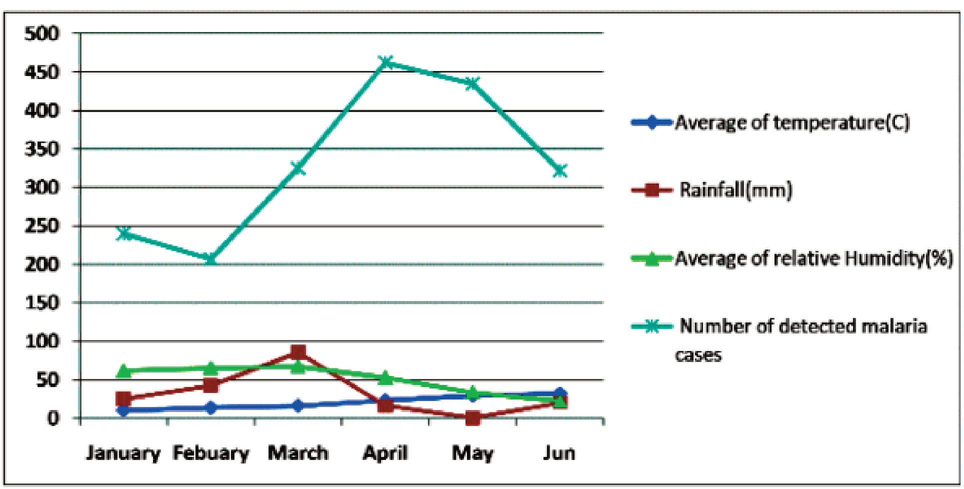

Figure-14: Mean number of detected malaria cases in relation to climatic factors in district Bannu.

Overall, 37(57.8%) breeding sites were positive for anopheles larvae, of which 259 anopheline larvae were sampled (Table-2). Also, 15(23.4%) habitats were positive for culicine and 75 larvae were collected from them. Besides, 12(18.7%) habitats were found to contain both types of larvae. The anopheline-positive habitats were found in agriculture lands (irrigation sites) and natural wetlands (marshlands).

The distance factor was counted because a mosquito uses the nearby population for the blood meal required for the development of eggs (Figure-12). The area with a distance of approximately 200 metres possessed highest number of habitats from the nearest house. The range of 200m included 21 habitats with anopheline-positive larvae. Besides, 3(4.6%) habitats were positive for culicine and 6(9.4%) were found to contain both. In the range of 300m, 10(15.6%) habitats were inhabited by anopheline, 6(9.4%) habitats of culicine larvae and 4(6.2%) for both. In distance of 90m very few habitats were found - 6(9.4%) for anopheline, 6(9.4%) for culicine and 2(3.1%) for both - because of human population activities like deforestation, farming, bathing and washing, which may disturb the potential breeding habitats of mosquitoes.

The larval density of 30(46.8%) breeding sites (3(4.6%) sites per village) have been determined from the 10 villages for the entire period of study. The larval breeding sites were visited twice-a-month and its larval density was subsequently noted. The climatic factors like the temperature of the area, rainfall and humidity effect was analysed on the larval density in breeding sites (Figure-13, 14). During January and February the average temperature in district Bannu was 12°C and the anopheline larvae were nearly absent in breeding sites or were present with a very low density with a dense vegetation or high canopy cover. Similarly, the average rainfall during these months was low and resultantly the low larval density has been found. The humidity has linear effect but due to low temperature the anopheline mosquitoes could not develop (Table-2).

As the temperature goes from low to medium the larval density also increases (16.1-23.25°C) during March-April (Table-3). The anopheline larval density also increases showing that the climatic factor during this time may be favourable for larval breeding and development. The malaria incidence was high as result of high vector density during this period of time.

Discussion

In order to plan and implement the effective malaria control strategy, the knowledge of the ecology of the anopheline mosquito's larvae is of utmost importance.[25] In this study the nature, type, occurrence, substrate type and vegetation were the main input variable determining the suitability of breeding sites for anopheline larvae. Due to increasing human population the anthropogenic activities are at high speed like livestock rearing, agriculture, deforestation and brick making.[26] High occurrence of anopheline larvae was present in temporary habitats and their low occurrence in permanent habitats which may be attributed to the presence of vertebrate and invertebrate predators and competitors in permanent breeding sites such as river fringes, some type of marshland and manmade ponds because they inhibit the density of anopheline larvae by feeding and other competition.[26] The permanent habitats present in the Mandan Parks (ponds) were found to contain low anopheline density or were absent. These findings are similar to the study of Yasuoka et al.[27]

The effect of climatic factor on malaria transmission is very significant (Table-4). The vector distribution in semi-humid tropical climate is high. For high (not high than 30°C) temperature and rain have positive effects on vector development.[28] This study indicates that the larval density in permanent habitats changed due to shift in climatic factor. During the month of January the larval density was low because of too low temperature. But as the climatic factors become suitable, the larval development also shifts to high as reported during the month of March-April. According to other studies the Anopheles occurred in the Serra Da mesa power plant Cerrado savannah region of Goias state was high during the rainy season.[29] The increased urbanisation and land uses are the main cause of the micro-climate change in district Bannu. The region is conflicted against war on terrorism and facing the burden of internally displaced persons of North Waziristan Agency. These factors may be the cause of positive shift in the bio-diversity of vector and hence high risk of malaria transmission.

The application of GIS in public health is a young line of research as is applied in this study. The malaria control campaign is always meant to be effective within the available resources. The accurate and timely information is necessary for the planning of malaria control and resource allocation. The computerised maps help to understand the malaria epidemiology and intervention measures.[31] Malaria is a vector-borne disease in western border areas of Pakistan like Bannu and may encounter the outbreaks. The GIS database of larval breeding sites, their nature and occurrence, rainfall, humidity and temperature may give rise to clear hotspots and hence malaria transmission risk can be easily implemented. Many features seem to affect the distribution of malaria transmission risk.[32] For example the land uses surrounding the population. The areas, where the crop plantation was the main occupation of the area were at more risk of malaria transmission. Because the breeding sites for mosquitoes were available easily due to unplanned agricultural practices. The human population density may also contribute to malaria transmission risk. The activities like construction, farming, bricking manufacturing, transport, poor drainage system, provide the favourable breeding sites for anopheline mosquitoes.[33] The availability and stability of aquatic habitats are affected by the topographic features of the study area. These results are similar to other studies,[34] as they found that the land use and topographic features affects the spatial distribution of larval habitats and human settlement and hence can have significant effect on malaria transmission risk.[35]

This study has highlighted the salient features of malaria vector anopheline larval habitats. The ecology of anopheline larvae has been determined in each village of the study area. The eight types of larval habitats have been characterised and GIS map has been provided that could be easily applied to estimate immature and adult density.[36] Thus the larval breeding site map in relationship to villages of district Bannu could be used to provide important information. This information could be easily used to allocate the resources for malaria control operations.

Conclusion

Marshes, irrigation channel margins, drainage pools and temporary rain-pools were the most favourable breeding places for anopheline mosquitoes. There is a need to carry out habitats-selective and time-specific operations during malaria-control campaigns. Also, there is need to carry out a study on physio-chemical analysis of water of larval breeding sites and their impact on anopheline larval density in District Bannu.

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