JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"STUDY OF CORRELATION AMONG DIFFERENT PHYSIOLOGICAL FACTOR TO THE LARVAL ECOLOGY IN PERENNIAL FRESH WATER BODY WITH REFERENCE TO BEEDPURA POND, MANAWAR, MADHYA PRADESH, INDIA."

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ABSRACT:

Mosquito constitutes a very important group of insects carrying vector for various disease. The availability of diverse water bodies supports their breeding. These breeding grounds which harbor the aquatic stages of mosquitoes transmit various parasites and pathogens causing human health hazards. Malaria still continues to be one of the most dreadful diseases of public health importance. It is transmitted via Anopheline mosquito vector which is more prevalent in tropic and subtropical region. WHO reports (2020) clearly mentioned that there are up to 50 million clinical cases of Malaria throughout the world annually. The present problem was therefore proposed with this particular aims and objectives to provide detail information of breeding habitats of Anopheline mosquitoes at Manawar Taluka of Dhar district of M.P. The study was conducted during the year 2021-2022, selecting the following breeding habitats for aquatic stages of Anopheline mosquitoes. The breeding grounds include cement tank, cesspool, ditches and one large pond "Beedpura" of Manawar Taluka of Dhar district of Madhya Pradesh.

KEYWORDS: Anopheles stephensi, Anopheles culicifacies, Malaria, breeding habitats, physicochemical, total dissolved salts etc.

INTRODUCTION:

Mosquito exploits almost all type of lentic aquatic habitats for their breeding. Larvae of Anopheline mosquitoes have been found to thrive in aquatic water bodies such as fresh water ponds, rice fields, stream and very few rivers(1). Many species of Anopheline mosquitoes prefer habitat with vegetation while some breeds in open water bodies (2). There are few species which breeds in tree holes and in remnants of tires. Some Anopheline species are known to be ground pool breeders (3). Although large number have been observed in gutter and domestic container. Saxena et al (1998) have reported earlier that Anopheline mosquitoes have been found to breed in clear water and fresh water of suitable pH, temperature and nutrients. Okogun (2005) have also noted that the range of pH of water (6.82 - 7.9) was found to be most suitable for female mosquitoes to lay their eggs.

Aquatic vegetation with optimum range of temperature, concentration of Ammonia, nitrate and sulphate have been found to effect the larval development and their survival.

Insecticidal control measures have been recognized as the most effective means of the vector control for which knowledge of larval ecology, physiochemical characteristics of the breeding grounds is very essential.

Mosquito's species differs in the type of aquatic habitats. The female mosquitoes prefer the breeding habitats based on local surroundings for their oviposition. The physicochemical condition of the water bodies and the presence of potential predator that influences oviposition, survival and distribution of important malaria vector species include total dissolved salts (TDS), organic and inorganic matters, degree of eutrophication, turbidity, presence and absence of aquatic plants, light and shade condition and pH of water (3). Understanding of these factors is very essential for the distribution pattern of a particular vector species. Therefore, these factors need to be studied, along with aquatic vegetation for the proper abudance and larval ecology.

Several studies have been conducted in the past few years to examine the relationship between physicochemical characteristics of breeding habitats and their correlation among different aquatic stages of Anopheline mosquitoes (4). *Anopheles stephensi* and *Anopheles culicifacies* are two important vector species of malaria in the state of M. P. Their abundance is positively associated with light and vegetation and negatively associated with the presence of potential predators.

The physical and chemical nature of water probably determinates the selection of breeding sites (5). This is one of the reason that in the present study, physicochemical characteristics of some breeding habitats of Manawar Taluka of Dhar district of M.P. was proposed to be investigated.

The use of urban water bodies in agriculture has become wide spread practices in India. This provides patches of water reservoir for the breeding of mosquitoes. The waste water as well as water locked areas also provide a reservoir for mosquito breeding habitats. Therefore, the waste water as well as irrigated land also to be considered for any control measures of vector borne diseases. A research publication in Thailand have clearly mentioned that successful larval control requires the ability to identify larval habitat and distinguished between the sites with high and low water pollution in a timely manner. Temperature and water quality such as salinity are the major contributor to attract the gravid female Anopheles mosquitoes for egg laying. The epidemiological and ecological data on Anopheline mosquitoes in the remote areas such as Manawar Taluka of Dhar district of M. P. have not been thoroughly investigated earlier. Therefore, the present study was proposed to investigate some breeding habitats to find out the relationship of malaria vector and larval ecology in relation to the physicochemical characteristics of water bodies. The role of aquatic vegetation in the distribution of larval ecology has also not been fully understood. The problem therefore, seems to be quite important in respect to the vector mosquitoes. Since mosquitoes spent most of its lifecycle in fresh water bodies. Therefore, understanding of these habitats, their ecology, types of vegetation in relation to the larval abundance with response to the environmental factors is an important aspect.

Adult female mosquito after blood meal selects certain aquatic habitat for oviposition and breeding.

Though, there is an extensive literature available on various aspects of mosquitoes breeding in the country, yet very few published reports on the relationship between water quality and vector breeding is available.

Therefore, for any control measures, the ecology of the plank tonic algae is very essential (6). The physicochemical characteristics of the aquatic environment directly influence the life of larvae in the aquatic breeding ground. The zooplanktons are considered as one of the most important linkage in aquatic food chain and play a major role in energy transfer. Therefore, population of zooplanktons with reference to the system energetic provides the key information for the management practices. Zooplankton concentration and distribution are very much sensitive to physical and chemical

changes in the water bodies. The knowledge of their seasonal quantitative and qualitative fluctuation has been considered essential for proper growth of the larvae(6).

Diptera represents one of the largest group of insects with more than 85000 species including some prominent vector of human health hazards (7). Prominent among them are mosquitoes which are placed under sub-order Nematocera and family Culicidae. More than 3100 species of mosquito belonging to 34 genera have been recorded under 3 subfamilies (8).

In India various species of Anopheles caries encephalitis, malaria and some viral borne disease. Larval ecology of malaria vector has been a neglected area of research. Entomologists have traditionally been reluctant to study larval ecology. One major reason for the lack of ecological study on Anopheline larvae is the difficulty involve with larval sampling from the aquatic habitat (9).

In India *Anopheles stephensi*, Anopheles *culicifacies*, *Anopheles subpictus*, *Anopheles annularis* and *Anopheles sundicus* are the major vector of malaria(10). Stagnant water bodies have more complex and tragic ecosystem in comparison to the running water bodies as they like self-clearing ability enhance readily accumulate greater quantity of pollutants. Increased anthropogenic activities in and around the water bodies damage the aquatic system and alter the physicochemical property of water.

The man is polluting and exploiting natural water resources at a large scale. The efforts to conserve these resources are present need. Factors that influence the sustainability of such lentic system are temperature, transparency, salinity, biogenic salts, dissolved gases etc. (11). Since, ponds are favorable habitats for a variety of flora-fauna and also used by the anthropogenic society, so its regular monitoring is necessary for control. Recently lot of work has been done on changing ecological behavior of ponds (12).

In the present study two important ponds of Dhar district were chosen for comparing the impact of biotic activities on physicochemical characteristics of ponds water. The study was performed during 2021-2022. Large number of studies covering a wide variety of ecosystems and organisms suggest that species richness tends to vary strongly with ecosystem production and habitat heterogeneity. This is particularly show with fresh water fauna.(zooplankton), which play a key role in preservation and maintenance of ecological balance and its basic study is wanting which is absolutely necessary (13).

Seasonal fluctuations of the zooplankton population are a well-known phenomenon and zooplankton exhibits a bimodal oscillation with a spring and autumn in the temperate lakes and reservoirs (14).

This fluctuation is greatly influenced by the variation in the temperature along with many other factors. Among several factors temperature seems to exhibit the greatest influence on the periodicity of zooplankton. Water temperature between 10 - 29 °C is suitable for zooplankton development(15). However, in shallow, tropical, perennial and seasonal ponds such as a regular food cycle can be seen. Thus in any aquatic ecosystem zooplankton not only take part in transferring food from primary to secondary level but also switch over conversion of detritus matter in to edible animal food.

By reviewing the existing literature in the relevant field and looking to the importance of the problem, it was thought important to investigate the ecology and bionomics of Anopheline mosquitoes at their early aquatic stages in a Beedpura pond of Manawar Taluka of Dhar district of M.P. with the following main aims and objectives:

To make a survey of permanent and temporary breeding habitat of Anopheline mosquitoes at Manawar.

To study the correlation among different physiological factor to the larval ecology in a perennial fresh water ponds of Manawar.

To observe the effect of aquatic vegetation on the female mosquito for fecundity and fertility up to the development of adult stages.

MATERIALS AND METHODS

Topography of the study area

The present study was carried out at Manawar Taluka of Dhar District of M.P. state in Central India. Manawar has a river named Man and surrounded by many cesspool and small pond. Beedpura (Manawar) has one medium sized pond which receives rain water and run off water from the fields. It doesn't receive any domestic effluents. Besides this pond, some temporary breeding ground of *Anopheles* mosquitoes was also searched during rainy seasons. It is dried after few months.

(ii) Sample Collection

The habitats selected under study is Beedpura pond and cesspool near Govt. College at Manawar Taluka on Dhar road. Water samples were collected from the pond and cesspool of Manawar. It receives direct sunlight. There are some species of fishes including *Mangur, Catla, Labio rohita*, and carp are found in the pond.

Sampling period – 2nd week of every month standardized at 10.00 am each day. Sampling done for tenure of 2 years viz. Dec 2021 to Nov. 2022., respectively.

Sampling apparatus – Net used for sampling of Zooplankton made up of 50 ml glass tube equipped with nylon micro filament and 75 µm mesh conical with specifications of 0.25 mouth diameter.

Counting – For planktons counting, the Sedgwick rafter cell was used. The S-R cell was held in stable position for 15 minutes in order to settle down planktonic mass. Then plankton on bottom of the S-R cell were counted, and further enumeration procedure performed by compound microscope. Organisms lying between two parallel cross hairs were counted as they passed on vertical line.

Number of plankton (Phyto and Zooplankton) in the S-R cell was derived using following formula (16).

$$\text{No species / } \mu = \frac{C \times 1000\ mm^3}{L \times D \times W \times 5}$$

Here, C = Number of organism counted

L = length of each strips (S-R cell length) in mm.

S= Number of Strips counted (mm)

D = Depth of each strips (mm).

W = Width of each strip (mm).

S = Number of strips.

Biological analysis and identification of the plankton was studied under microscope and identified with the help of standard reference (17).

Phytoplankton species richness diversity and ever ness were carried out using the standard methodology(18) .

Eight species of aquatic insects were also collected with the help of nets from the habitats.

(iii) Method for Physicochemical analysis

The analysis of water sample was done according to the standard method of APHA (2021). Freshwater bodies in Beedpura pond and second is cesspools, in front of Govt. college of Manawar. Physicochemical parameters analyzed according to standard methodology (19).Physicochemical characteristics of mosquito breeding habitats were examined (10).

Temperature- Air and water temperature noticed in centigrade by mercury thermometer.

pH– Potential of hydrogen ion concentration was recorded by electronic pH meter (Systronic Model 324).

Sunlight – Direct sunlight received or not received by water bodies was noted.

Water Flow- it was evaluated in terms of slow or fast moving stagnant water.

Color- It was noticed by visual observations.

Free CO_2 – It was analyzed using NaOH titrate and phenolphthalein indicator method.

Alkalinity- Carbonate and Bicarbonate alkalinity were determined titrametrically using phenophenol indicator and methyl orange indicator, respectively.

Nitrate- Analyzed by nitrate electrode method using phenol di-sulphonic acid to form a nitro derivative nitrate. Nitrogen concentration is calculated with the help of standard curve.

Chloride - Chloride present in water was estimated titramatrically by Mohr's(2010) 's method of Argentometric and mercuric nitrate.

Calcium – EDTA titration method was applied for calcium estimation.

Hardness – EDTA titration method was applied for hardness.

Dissolved Oxygen – It was determined by modified Winkler's method.

(iv) Mosquito sample collecting methods.

Larval collection of the Anopheline mosquitoes were carried out along with the margin of the water bodies (Pond and Cesspool) using an 8 cm diameter and 240 ml capacity long hand dipper. In a single collection five dipper sample were taken from a 1 $_M^2$ area. Total 10 samples were collected covering different locations of the pond. The collected larvae along with the water samples were brought to the laboratory and kept in the insectary and separated instar wise to larvae and pupae and their numbers were counted manually by a net fitted hand apparatus and noted down the number.

(vi) Biostatistical Methods–

Biostatistical method and the correlation between the samples was done by ANOVA and Student 't'-Test. Pearson's correlation was used to determine the relationship among physicochemical parameters and larval ecology.

Diptera is one of the largest order of insecta and includes many flies as medical importance and structurally well-developed among insects.

Physicochemical characteristics of the breeding grounds

Monthly sampling was taking in 3 different seasons round the year and the results were tabulated in below Tables. It was noticed that there is a seasonal fluctuation in physicochemical characteristics of both the breeding habitat Beedpura pond and cesspool.

Physicochemical characters and larval density

During the course of present study, temperature of air and water, color, pH, dissolved oxygen, nitrate, chloride, salinity, free Ammonia and free CO_2 parameters were noticed in Beedpura pond and their correlation was established with the larval density in both the habitats. The pH value varied from 6.8-8.2 during 2008-09 in one breeding habitat whereas 6.5-7.8 in cesspool breeding habitat thereby showing the alkaline nature of the water bodies, except in extreme summer months, it becomes slightly acidic. Alkalinity was found to be directly correlated with the larval density of Anopheline mosquitoes in both the habitat. Maximum 125 to 200 larvae were recorded in per dip of sampling of water. As regards the dissolved oxygen (D.O.), it was always above 4.5 ppm in both the habitats during the study period which goes maximum up to 8.8 in Beedpura pond. During summer months the alkalinity was recorded maximum 202 ppm during 2021-22. In the summer months, hardness of water (T.D.S.) hardly exceeds 200 ppm and salinity is also increased maximum up to 3.7 ppm which indicate that both the habitats are the reservoir of fresh water. Similarly, nitrate, chloride, calcium, free ammonia were also observed during the study period. Chloride content of the water varied between 28.0 to 55.5 during 2021-22 in Beedpura pond. Calcium level in both the habitats never exceeds 26.2 ppm. This is because of both the habitats being fresh water breeding grounds. The number of larvae were found maximum (125-200) during monsoon months. This was followed by 110-150 larvae in summer months.

Among phytoplankton, *Myxophyceae* was the most dominating among the three families. It was represented by *Anabaena*, *Oscilattoria* and *Microcystis* namely. As reported in table (16) *Microcystis* accounted the maximum number (37049 cells/ lit.) followed by *Oscillatoria* (17978 cells.lit.). The Bacillariophyceae was represented by two genera only, *Cyclotela* and *Navicula* , whereas the Euglenoidae was represented by two genera only. These are *Euglena* and *Zygnema.* It was noticed that negative correlation of planktonic algae was found with CO_2, alkalinity, hardness (TDS) and dissolved oxygen whereas positive correlation was noticed with nitrate, chloride, calcium, air and water temperature with the planktonic algae

RESULT

The results mentioned in table shows the Pearson correlation matrix of different physicochemical variables and planktons recorded from the Beedpura pond and cesspool. Total 15000 *Cyclotela* and 22300 *Navicula* cells were noticed among Bacillariophyceae (20).

Seasonal variation in plankton's diversity was recorded in highest number in December to March season.

Bio statistical analysis (Correlation and Regression) of physicochemical parameters.

Among physicochemical parameters, temperature was found to be negatively correlated with Euglenoidae whereas positively correlated with Bacillariophyceae and Myxophyceae. The following physicochemical factors were found to be positively correlated with mosquito population. These parameters include total hardness, nitrates, chlorides, calcium and dissolved oxygen

Temperature of water and air seems to be positively correlated with larval density. As the temperature increases larval density from both the habitats were found to be increased. pH of water were also found to be positively correlated slightly, alkaline pH shows higher larval population ($r = -0.7968$ and $P < 0.05$). The dissolved oxygen was negatively correlated when the dissolved oxygen is less, the larval density was much but as it goes beyond 6.5, the larval density got decreased in both the habitat ($r = -0.0073$) as shown in table below.

From the results in table it appears that alkalinity is positively correlated with larval population when the alkalinity is 120 ppm the larval density was more during winter months. When it increases to 202 ppm during summer, the number of larvae got increased. It seems from the results that not a single factor is correlated with the larval population. There are number of factor which influences the larval ecology in the breeding habitat. Hardness of water shows a positive correlation with the larval ecology ($r = 0.693$ and $p < 0.05$). Calcium ,chloride and nitrate content in the breeding habitat showed positive correlation with the larval ecology ($r = 0.0801$, $r = 2.19$, $r = 0.2978$, respectively) and ($p < 0.05$).

During plankton sampling eight species of aquatic insects from Beedpura breeding habitats were also recovered. These insects are very much significant as many of them pollution indicator, some are larvivorous such as *Lethocerous indicum*), *Macromia magnifica v instar Macromia magnifica ecdysis Macromia magnifica adult Macromia magnifica iv instar nymph Notonecta glauca Renetra elongata, Daphnia resticum*.

SEASONAL VARIATIONS OF PHYSICOCHEMICAL PARAMETERS IN RELATION TO MOSQUITO BREEDING HABITAT OF BEEDPURA POND DURING 2021-2022.

Physicochemical parameters	Seasons		
	Monsoon	Winter	Summer
Temperature Air °C	28.0	20.2	35.8
Temp. Water °C	26.5	17.0	30.5
Sun light	Intermittent	Direct	Direct
Water flow	Slow	Stagnant	Stagnant
Color	Light green	Green	Brownish
Odor	Absent	Absent	Present
pH	7.5	8.2	6.8
Alkalinity in ppm.	138	135	180
Salinity in ppm.	2.1	2.5	3.7
D.O. in ppm.	6.0	8.8	4.5
Free NH3 in ppm.	0.01	0.01	0.01
Free CO2 in ppm.	1.5	2.5	6.5
Total Hardness	125	202	198
Nitrate in ppm.	0.30	0.38	0.55
Chloride in ppm.	34.2	43.5	65.2
Calcium in ppm.	20.5	24.4	26.0
Larval density	125-200 per dip	65 per dip	80 - 110 per dip

The mosquito breeding habitat study in relation to the aquatic vegetation, planktonic population and physicochemical characteristics of the present study concludes –

The study was conducted in a fresh water, perennial pond and a temporary water cesspool at Manawar in 3 different seasons round the year during 2021-22. The data of the breeding habitat was investigated and recorded which showed slightly alkaline pH 7.8 that is very much positively correlated with the larval density. Temperature of the water and air, pH and alkalinity were found to directly correlated with the larval density and proliferation in both the breeding habitats. Total

hardness, Nitrate, chloride and calcium contents was found to be positively correlated with the larval density ($p < 0.05$). Dissolved oxygen is negatively correlated with the larval density in both the breeding grounds. The factors like free ammonia, free carbon dioxide ($r = -0.0436379$ and $p < 0.05$) and salinity are negatively correlated. The mean larval density of *Anopheles stephensi* in this study was higher in slightly turbid and shallow aquatic habitat than in turbid and relatively deep aquatic habitat. ($f = 16.87$, $P < = 0.85$).

DISCUSSION

The study revealed that phytoplankton and zooplankton provide a food web in the pond ecosystem giving positive correlation to the larval density (10). However, some member of blue green algae (Cyanophyceae) when eating by the mature larvae choked the alimentary tract and this may be detrimental and can be exploited as a control measure for mosquito vector (21). Some aquatic insects having larvivorous potential were also recovered from the breeding habitats which may be used as biological control agents. During the present study 19 aquatic plants have been identified and their role in mosquito abundance was discussed. Further investigation on the species diversity, physical and chemical habitat characteristics and impact of water holding capacity of the soil need to be generate detail database line for future researches.

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