

Spatial distribution and characteristics of *Anopheles* larvae breeding places and their relation to larval density in Bulukumba

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Abstract. The World Health Organization estimates that 207 million cases of malaria occurred globally in 2012 had resulted in 627 thousand deaths. Bulukumba District is one of the malaria-endemic areas as case reporting every year. The study aims to determine the spatial distribution and analyse habitat characteristics and a density of *Anopheles* larvae in Bulukumba Regency. The method of the research was observational studies by ecological survey design. The 103 of breeding places situated in 5 sub-districts of Bulukumba were observed namely physical, biological, and chemical characteristics. Distribution of species was expressed on spatial mapping of GIS. The spatial distribution of *Anopheles* larvae was at a distance of 0-1000 m from homes of patients that spread in 5 seconds. Physical environmental factors had significant effect on larval density ($p=0.045$), alongside with water temperature ($p=0.017$), biological environmental factors as the presence of aquatic plants ($p=0.010$) and larval predators ($p=0,000$), and a chemical environmental factor that was pH ($p=0.031$). Multivariate analysis using linear regression test showed that larval predators were the most influential variable on larval density ($p=0,000$). In conclusion, physical, biological and chemical characteristics affect the density of *Anopheles sp* larvae. The environmental management is needed, especially the breeding habitat of *Anopheles sp* larvae by sowing predatory fish seeds in permanent habitat types.

Keyword: *Anopheles* larvae, larval

1 Introduction

Malaria is still a global public health problem. Nearly half of the world's population is at risk for malaria and causes one million deaths [1,2]. Malaria in pregnant women increases the risk of low birth weight babies, infant mortality, abortion, anemia, and even death in pregnant women [3].

The Bulukumba Health Office records the number of malaria cases based on API values from 2009-2014. API in 2009 amounted to 4.29, in 2010 there was an increase in API value to 5.3, in 2011 the API value decreased to 0.29, 2012 and 2013 API values decreased by 0.13 and 2014 by 0.06%. The decline in the value of API is due to the existence of a global fund program in controlling malaria [4].

Asniar in 2012 concerning confirmation of entomology of malaria cases in Bulukumba Regency there were 6 species of *Anopheles* mosquitoes that have the potential as transmission vectors namely: *An. barbirostris*, *An. Vagus*, *An. subpictus*, *An. Indefinite*, *An. hircanus* and *An. Kochi*. From the type of mosquito *An. barbirostris* and *An. subpictus* is a malaria vector in Bulukumba District [5].

The presence of larval habitat is a ubiquitous factor for the occurrence and maintenance of vector population densities, which are directly involved in the rate of malaria spread. The main Neotropical vector is the *An. barbirostris* and *An. subpictus* mosquitoes.

Environmental factors have a very important role in the role of health as Anopheles habitat research conducted by Suwito et al. on the relationship of climate, the density of Anopheles mosquitoes and the incidence of malaria showed that mosquito density was associated with an increased incidence of malaria ($P = 0.021$) [6].

Environmental health research will get interesting if using geospatial technology. The fundamental concept of environmental health is related to the place of the life of humans themselves, which are elements of the environment, such as climate, soil, air, and insects [7].

Spatial analysis and distribution are one of the area-based disease management methodologies. Analysis of malaria, for example, considers the number of patients in an area at a certain time by taking into account the variables of rainfall, humidity, vector density, altitude, the location of mosquito breeding sites, and various other variables in an ecosystem [8].

The use of the GIS application and the spatial analysis method makes it easy to control the mapping of malaria vector measures to meet the specific needs of the field team as well as supervisors and program managers in assisting malaria control programs and understanding the spatial distribution of disease [9], [10].

The purpose of this study was to determine the spatial distribution and habitat characteristics with the density of Anopheles larvae in Bulukumba Regency.

2 Method

The research design was observational with the design of ecological studies through a cross-sectional approach which aimed to determine the relationship between several variables of the study with environmental samples in the form of larval habitats.

The research was carried out in Bulukumba District. The population of all habitat of Anopheles mosquito breeding. While the research sample is the breeding habitat of the Anopheles mosquito, the sampling method is carried out by purposive sampling. Namely all samples found when conducting research that can be reached and can be carried out. The study sample was at a distance of 100-1000 meters from the case house.

The instruments used in this study were observation sheets, survey equipment, and field laboratory equipment in the form of injuries, pipettes, larval containers, net larvae, gutters, digital salt meters, GPS, pH meters, label paper, and writing instruments.

Determination of larvae and density is done by scooping up a body of water, where for a narrow water body is done 10 times while for a broad water body is done more than 10 times. If the results of the wound contain larvae, the species is observed for the larvae; if the larval position is parallel to the surface of the water, then the larvae are Anopheles.

Analysis of data from observations and entomology surveys was carried out with the univariate analysis in the form of tables and graphs with narration as an explanation, bivariate analysis to determine the effect and correlation between variables, and multivariate analysis to determine the strength of influence among variables.

Identification of Anopheles larvae species by collecting them in a sample bottle and rearing them to become adult mosquitoes, then preserved in Effendorf tube containing silica

gel and cotton. Calculation of larval density is done by formula. Other paragraphs are indented (BodytextIndented style).

$$density = \frac{\text{number of caught}}{\text{captured number of larvae}}$$

3 Results

The type of research used in this study is observational with the design of ecological studies. Through the environmental approach, this study looked at the breeding place characteristics with the larval density data of local malaria sufferers. Sampling is set at a 500-1000 meter radius from the patient's home.

3.1 Spatial distribution of *Anopheles* larvae

The study area is a foot mountainous area, namely Rilau Ale, and Bulukumpa District and part of the hill and coastal areas are Bontotiro District, Bontobahari District, Ujungbulu District, and Ujung Loe District. Geographical conditions from 6 sub-districts are different. This research was conducted by observing the habitat of larvae of *Anopheles* sp. within a radius of 500-1000 meters from a malaria case. Furthermore, the positive and negative habitat of *Anopheles* sp. Larvae were determined. And the larval density of each positive habitat for *Anopheles* sp. larvae.

Environmental characteristics were also measured to analyze the effect on larval density. Figure 1 shows as many as 103 habitats observed, in various research districts. Distribution of cases and various types of habitat types within a radius of 500-1000 meters. The case buffer aims to describe a positive or negative habitat type that is within a radius of 500-1000 meters. Figure 1 shows a small portion of positive habitats located around the case house with high rice field habitat types found positive for *Anopheles* sp. Larvae.

The species of larvae that have been identified in this sub-district are quite varied, namely *An. vagus* mostly in the type of rice field habitat, the rest is found in puddles and footprints, *An. Hyrcanus* is found in the rice field type habitat, *An. subpictus* is found in paddy and pond type habitats, *An. Indefinite* is found in rice field type habitats and *An. barbirostris* is found in river type habitats, swamps and rice fields. Distribution of *Anopheles* species can be seen in Figure 1.

Distribution of *Anopheles* sp. Larvae. Varies between different sub-districts in Bulukumpa. For larvae *An. barbirostris* can be found in highland and lowland areas. Larva *An. barbirostris* is found in Bulukumpa district, Rilau Ale, Ujungbulu and Ujung Loe with habitat types in the form of rice fields, rivers, pools, and swamps. As for the larval distribution of *An. subpictus*, where the larva *An. subpictus* is dominant in Bontobahari, Ujung Loe and Ujungbulu. This indicates that *An. subpictus* is found in coastal areas with pond habitat types. Distribution of larval species *An. Hyrcanus* and *An. Indefinite* is spread in Bulukumpa and Rilau Ale districts with the dominant habitat in rice fields, while for larvae *An. Vagus* is spread throughout the study area.

The public health center which is the location of research is the Public Health Center (PHC) Cable in Ujungbulu District, PHC Ujung Loe in Ujung Loe District, PHC Bontotiro and PHC

Batang in Bontotiro District, Bontobangun PHC in Rilau Ale and PHC Tanete Districts in Bulukumpa.

Table 1 shows the distribution of Anopheles larvae based on the PHC region, where larvae *An. barbirostris* is distributed in PHC Caile, Bontobangun, Tanete, and Ujung Loe, larvae *An. subpictus* is distributed in PHC, Bontobahari, Bontotiro and Ujung Loe. Larvae *An. Vagus* is spread in PHC Caile, Bontobahari, Batang, Tanete, Bontobangun, and Ujung Loe. Larvae *An. Hyrcanus* and larva *An. indefinite* is spread in PHC Tanete and Bontobangun.

Table 2 shows the distribution of breeding place for Anopheles larvae, where the larvae of *An. barbirostris*, *An. Vagus*, *An. Hyrcanus* and *An. Indefinite* dominant was found in rice fields while larvae *An. subpictus* is dominantly found in ponds and ponds. Table 3 shows the breeding place characteristics of each type of Anopheles larvae, which consists of physical environmental characteristics, biological environment, and chemical environment.

Table 4 uses the crucial wails test to see the difference in density between permanent and non-permanent breeding places where there is a significant difference with $p = 0.045$, table 5 shows the significant effect between water temperature and larval density with $p = 0.017$ and $r = 0.235$ which means the higher the water temperature, the higher the larval density. Table 6 shows the significant difference between aquatic plant and larval density with $p = 0.010$. Table 7 shows that there is a significant effect between larval predators and larval density with $p = 0,000$ and $r = 0.423$, which means that breeding places that do not have larval predators are higher in the density of Anopheles larvae.

Table 8 shows the significant effect between water pH and larval density with a value of $p = 0.031$ and the value of $r = 0.213$ shows that the higher the pH of the water the higher the density of the Anopheles larvae. Linear regression test as in Table 9 shows that larval predators are the most influential variable on the density of Anopheles larvae with $p = 0,000$. The adjusted square value is 0.227, which means that the larval predator gives an effect of 22.7% on larval density, and other variables influence 76.3%. Beta coefficient values indicate that larval predators can predict larval density with a correlation of 0.340

4. Discussion

This study found 6 cases of case houses, namely 2 point case houses in Bulukumpa District, and 4 points in Bontobahari District. From the home point of the case, the researchers found that they observed potential habitat for the breeding of Anopheles sp. Larvae. In this study, focused habitats were defined as habitats within a radius of 500 meters from malaria cases with consideration of mosquito flying distance, which is very limited in the range of 400 meters. The transmission of transmission develops widely because several variables that influence one of them are population mobility activity [11].

The study found that in Bulukumpa and Bontobahari districts where after spatial analysis with buffer method showed that at a distance of 500 meters from the house of malaria patients there was a positive habitat for breeding of Anopheles sp larvae so that at a distance had a strong influence as the transmission of malaria spread. This is relevant to Rohani et al. (2010) research, showing the relationship between the distance of a malaria patient's house and the breeding habitat of Anopheles sp larvae as seen from the distance of a case house with a positive habitat. This shows a strong relationship between the distance of breeding habitats and transmission of malaria spread [12].

Distribution of various types of Anopheles larvae which become malaria vectors in Bulukumba Regency, namely *An. barbirostris* and *An. subpictus* shows that *An. barbirostris* is predominantly found in Bulukumpa, Tanete and Ujung Loe Districts with habitat types in the

form of rice fields, rivers, and swamps, while *An. subpictus* is predominantly found in Bontobahari and Ujung Loe Districts with habitat types in the form of ponds.

An. barbirostris and *An. subpictus* which is a potential vector on the island of Sulawesi. *An. barbirostris* is generally found in hilly and rice fields while *An. subpictus* is found in coastal areas although it does not rule out the possibility that *An. barbirostris* can be found in coastal areas and *An. subpictus* likes rice fields.

Habitat characteristics *An. barbirostris* such as rice fields, rivers, and swamps are found in highland areas in Bulukumpa and Rilau Ale Districts and lowland areas such as Ujungbulu and Ujung Loe Districts.

The different types of mosquito species found are also related to the topography of a region; this can be seen in Figure 1 about the type of species distribution in Kab. Bulukumpa that *An. barbirostris* is found at altitudes ranging from 8-448 mdpl which indicates that *An. barbirostris* can be found in lowlands and highlands.

This is relevant to other studies, namely *An. barbirostris* has also been found to be associated with higher altitudes, paddy fields, shallow water depths, higher water temperatures, high pH and salinity, and an average distance further from human habitation [13],[14].

Research conducted by Ndoen et al. (2010), shows that *An. barbirostris* in the area of Central Java is more commonly found in areas in the highlands while in the East-West region (NTT) more are found in coastal areas and *An. barbirostris* can be found at an altitude of 0-800 m. *An. subpictus* was found in coastal areas, namely in Bontobahari with habitat characteristics in the form of ponds and Ujung Loe also found in rice fields at altitudes of 8-100 m above sea level. This is relevant to the research conducted by Ndoen that *An. subpictus* relates to lagoons, brackish water, and mangrove forests that receive direct sunlight [15].

An. subpictus in the area of Central Java was found in hilly rice fields, while in the East-West region it was found dominantly in coastal areas. This shows that the topography of the region influences the spread of *Anopheles* sp species.

Topography has a strong influence on the presence of the *Anopheles* mosquito where the topography shows that *An. barbirostris* can be at any height while *An. subpictus* is predominantly found in coastal areas such as research conducted by Ndoen et al. while *An. subpictus* at an altitude of 0-200 m [15].

An. vagus and *An. indefinitus* is found in the highlands and lowlands, namely in the type of habitat in the form of rice fields. *An. vagus* and *An. indefinitus* is not found in pond habitats. For *An. hyrcanus* is found in mountainous regions with the majority of habitat types in the form of rice fields.

4.1 Physical environmental characteristics

The characteristics of the physical environment that are the research variables here are water temperature, breeding habitat types, water depth, sunlight intensity, water flow, and habitat properties. The results of the bivariate analysis of physical environment variables that influence the density of *Anopheles* larvae include habitat type and water temperature.

In nature, *An. barbirostris* can inhabit larvae such as small ponds, swamps, and rice fields. Whereas in South Lampung, *An. barbirostris* breeding place is found. *An. barbirostris* is found in *An. barbirostris* breeding sites. *An. barbirostris* in rice fields with moderate sunlight intensity, in Manggala, North Lampung, *An. barbirostris* larvae were found. *An. barbirostris* in the rice field with a water pH between 4.5-7.0. In Sikka Flores larvae. *An. barbirostris* are found in flowing rivers and lagoons with a salt content of 0.2-10.4%. In general, the habitat favored by *An. barbirostris* larvae of sunlit water bodies,

such as lagoons, ponds, slow running waterways, along river banks, and rice fields and vegetation overgrown with vegetation [16].

Research that supports habitat characteristics of *An. barbirostris*, including research on dominant malaria vectors in the Asia Pacific region, shows that larval habitat is *An. barbirostris* has a type of brackish water habitat that is low in salinity, swamps, artificial lakes, rice fields, irrigation channels, and ponds with a correlation value = 0.866 [17].

Water temperature affects larval proliferation. In general, larvae of *Anopheles* mosquitoes prefer high temperatures when compared to the type of cuisine. That is why more *Anopheles* species are encountered in the tropics.

The hatching time of *Anopheles* mosquito eggs is strongly influenced by the temperature of the water at the breeding site. The higher the water temperature, the shorter the time. Water temperature correlates with the larval density of *An. barbirostris* in Ujung Loe with average larvae *An. barbirostris* was found at the water temperature that fulfilled the requirements; the results of multivariate analysis and analysis showed that the water temperature had the strongest influence on the larval density of *An. barbirostris* in Ujung Loe District is compared to variables. This is in line with research conducted by Christiansen et al. (2014), regarding the relationship of temperature to larval propagation shows that the optimal temperature for larval development of *An. gambiae* between 23-31^oC. Likewise with research by Rohani et al. (2010), about habitat characteristics and mapping of *An. maculatus* in Malaysia shows water temperature in each of the studied habitats 21-31^oC [18], [12].

4.2 Biological environmental characteristics

Research shows a significant relationship between aquatic plant and a density of *Anopheles* larvae. This is consistent with research by Kevin A and Caillouët (2008). Water plants in the breeding site play a role in the presence of *Anopheles* larvae. This is because the aquatic plants can function as a place to disinfect mosquitoes when on the surface of the water [19].

Algae and decaying water plants on the surface of the water that spread widely and gets direct sunlight greatly affect the proliferation of larvae, this is caused by microfauna and microflora as food ingredients for many larvae gathered around the plant.

Water plants or vegetation found in the larval breeding habitat of *An. subpictus* in the form of moss and grass. The existence of vegetation or aquatic plants can increase larval density because water vegetation becomes a hiding place for larvae from predators and protects the habitat from direct sunlight to reduce water temperature. The presence of predators affects the density of *Anopheles* larvae. Research by Michael El al (2005) with a value of $p = 0.040$ after logistic regression analysis, there was a predator influence on the presence of *Anopheles* larvae. This shows that there are more predators, so the density of larvae decreases. In the research location, the majority of breeding places have no predators [20].

4.3 Chemical, environmental characteristics

The degree of acidity (pH) is used in the regulation of respiration and enzyme systems in the body of mosquito larvae. In this study showed a significant relationship between pH and density of mosquito larvae. This is consistent with the study by Paulo et al. (2016) that the pH favored by mosquitoes ranged from 6-9 with a p-value of 0.008 at *An. darling* [21].

5. Conclusion

Physical environmental characteristics of larval predators are the most influential variables on the density of *Anopheles* larvae. The distribution of *Anopheles* larvae found in this study was in the form of *An.barbirostris*, *An.subpictus*, *An.vagus*, *An.hyrceanus* and *An. indefinitus*. It is suggested that cooperation between the Bulukumba District Health Office and the Public Works Agency be needed to improve the irrigation system, especially for temporal habitats to overcome the breeding habitat of *Anopheles* sp larvae.

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