ECOLOGY AND DISTRIBUTION OF MOSQUITO LARVAE IN THE INLAND HABITAT OF SOUTH SULAWESI, INDONESIA

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ABSTRACT

The ecology and distribution of mosquito larvae have a significant effect on mosquito populations and in arbovirus transmission. There is no information on the ecology and distribution of mosquito larvae in the inland area of South Sulawesi, Indonesia. This study aimed to examine distribution and ecological of mosquito larvae that affect the survival of arbovirus vectors in the inland area of South Sulawesi. Larvae were obtained in eight locations of the South Sulawesi region. The collected samples were examined in the Entomology Laboratory, Faculty of Medicine, Hasanuddin University, South Sulawesi, Indonesia. Larvae were counted, separated by genus and identified to the species level. The physical parameters of the water in the breeding sites (pH, temperature and salinity) and the ambient relative humidity were recorded. Data were analyzed using IBM SPSS version 24 and the spatial distribution of larvae was mapped using ArcGIS version 10.5. Results showed that there were 3,803 larvae consisting of *Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus, Cx. vishnui, Cx. tritaenorynchus, Cx. gelidus,* and *An. vagus*. The physical parameters of the water habitat for mosquito larvae range from a temperature of 24.5°C-34.2°C, water pH 6.3-7.5, humidity 67.8-89.0, and salinity 0 ppt. We found that mosquito larvae abundant had a significant relationship (P=0.00) with pH, salinity, temperature, and humidity. Results suggest that the abundance of arbovirus vectors in the inland of South Sulawesi can be controlled by vector control programs.

Keywords: Arbovirus, mosquito, inland area, South Sulawesi

ABSTRAK

Ekologi dan taburan larva nyamuk mempunyai kesan signifikan ke atas populasi nyamuk dalam transmisi arbovirus. Masih tiada maklumat ke atas ekologi dan taburan larva nyamuk ini di pedalaman Sulawesi Selatan, Indonesia. Kajian ini bertujuan untuk menilai taburan dan ekologi larva nyamuk bagi menilai jangka hayat vektor arbovirus di pedalaman Sulawei Selatan, Indonesia. Sampel yang dikumpul telah diperiksa di Makmal Entomologi, Fakulti Perubatan, Hasanuddin University, Selatan Sulawesi, Indonesia. Larva dikira, diasingkan ke peringkat genus dan dicamkan ke peringkat spesies. Parameter fizikal untuk air yang digunakan untuk tempat pembiakan (pH, suhu dan kemasinan) dan kelembapan relatif ambien telah direkodkan. Data telah dianalisis menggunakan IBM SPSS version 24 dan taburan ruang larva diplotkan menggunakan ArcGIS versi 10.5. Hasil menunjukkan sejumlah 3,803 larva merangkumi *Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus, Cx. vishnui, Cx. tritaenorynchus, Cx. gelidus,* dan *An. vagus*. Parameter fizikal pada habitat air untuk larva nyamuk suhunya di 24.5°C-34.2°C, pH air 6.3-7.5, kelembapan 67.8-89.0 dan kemasinan pada 0 ppt. Kelimpahan larva nyamuk menunjukkan hubungan yang signifikan (P=0.00) ke atas faktor pH, kemasinan, suhu, dan kelembapan. Kelembapan vektor arbovirus di pedalaman Selatan Sulawesi dapat dikawal melalui program kawalan vektor.

Kata kunci: Arbovirus, nyamuk, kawasan pedalaman, Selatan Sulawesi

INTRODUCTION

Mosquitoes are vectors of arboviruses and parasites such as malaria, chikungunya, dengue fever, and Japanese encephalitis (Ratnasari et al. 2020). In South Sulawesi, the acute infection of malaria, chikungunya, dengue hemorrhagic fever, and Japanese encephalitis were 0.13, 7, 19.96, and 52.3%, respectively (Arif et al. 2020; Ministry of Health Indonesia 2018; Widarso et al. 2002). Arboviruses and parasites transmitted by mosquitoes are associated with mosquito life-supporting habitats, one of which is inland areas far from the coast.

The inland portion of South Sulawesi is primarily used as agricultural land and settlements by the locals, and both places support mosquito habitats. According to Hamidun et al. (2021) ecological habitat of mosquito larvae in residential area and landscaping nursery area. Mosquito larvae can be found in forests and non-forest (Khariri, 2018). Mosquito larvae can be found on the banks of rivers (Chaiphongpachara et al. 2018). Mosquito larvae were related humadity, rainfall, dan temperature, precipitation (Drakou et al. 2020; Kesetyaningsih et al. 2018).

Larvae of *Anopheles* sp. can be found in rice fields, puddles, and irrigation canals (Inunggita et al. 2019; Robert et al. 2002). According to Ratnasari et al. (2021), the breeding places of *Ae. aegypti* can be found in bathrooms, used buckets, old tires, flowerpots, trash cans, paint cans, and water tanks. Meanwhile, Augustina et al. (2021) found that the habitat of *Culex* sp. was in used pots, swamps, sewers, and canals.

Ecological surveys of arbovirus and parasitic vector habitats are ineffective in inland areas due to various natural and artificial mosquito breeding sites. Therefore, many different mosquito habitats pose challenges to controlling and eradicating arboviruses and parasites. In addition, there is no information on the ecology and distribution of mosquito larvae in the inland area of South Sulawesi. The aim of the study analyzed mosquito larvae in inland areas, which have the potential as arbovirus and parasite vectors.

MATERIALS AND METHODS

This observational study used simple random sampling to collect the distribution of mosquito larvae and habitat ecology in the inland region of South Sulawesi. Simple random sampling is a random sampling technique without distinguishing those in the population.

Study Areas

Mosquito larvae were collected in inland South Sulawesi from January 2018 to December 2019. Should be eight districts include the district of this city were chosen for sampling (Table 1). The location is chosen based on the ease of access and the number of dengue cases (Figure 1).

Table 1. Latitudes and longitudes of the study locations in the inland area of South Sulawesi, Indonesia

District/City	Sub-District	Lattitude	Longitude
Makassar	Biringkanaya	5°03'35.6" S	119°28'8.37" E
Maros	Marusu	5°03'71.5" S	119°49'91.6" E
Gowa	Pattalassang	5°12'32.3" S	119°33'26.5" E
Takalar	Palombangkeng	5°24'14.0" S	119°30'02.9" E
Jeneponto	Bontoramba	5°35'28.0" S	119°40'06.2" E
Bantaeng	Sinoa	5°29'54.8" S	119°55'27.3" E
Bulukumba	Rilau Ale	5°26'34.9" S	120°12'24.0" E
Pangkep	Balocci	4°53'49.4" S	119°38'13.2" E

Figure 1. Map showing the eight districts of the inland area of South Sulawesi with the selected sampling location shown with pin marker

Insect Sampling

Sampling was carried out from morning to noon (8:00 a.m. to 12:00 p.m. local time) by searching for breeding habitats for mosquito larvae. A Pasteur pipette with 10 cm diameter filter was used for small samples, whereas a dipper was used for large numbers of larvae. In addition, all mosquito larvae in containers with water were transferred to sample bottles, labelled, and the water's physical parameters were measured. The coordinates for each location were recorded using a Garmin GPS eTrex-10 series. Collected samples were brought to the Entomology Laboratory of Hasanuddin University for identification and analysis. A sampling at eight locations based on variations in densely populated inland areas rarely touched by mosquito eradication programs. Consideration of location selection based on easy access to span that has high data on cases of dengue hemorrhagic fever.

Physical Parameters Data Collection

Measurement of physical parameters in larval samples included pH, temperature, humidity, and salinity using digital tools. The parameters of pH and temperature were measured using an instrument (Yieryi-3508), a salinometer (Lutron YK-31SA), and a hygrometer (AMF051).

Species Identification

Mosquito larvae were counted and grouped based on the sampling location. The mosquito larvae were identified using a stereomicroscope and taxonomic identification key by Rueda (2004), O'Connor and Soepanto (1999) and Ministry of Health Indonesia (2017).

Data Analysis
The chi-square analysis assessed the number and percentage of larvae in each habitat. The Pearson correlation test examined the relationship between the number of larvae in each location and physical parameters. Finally, the spatial distribution of larvae was mapped using the ArcGIS 10.5 application.

RESULTS AND DISCUSSION

A total of 3,803 larvae were collected during field observations consisting of three genera and seven species with include *Ae. aegypti* (n=2,059), *Ae. albopictus* (n=540), *Cx. quinquefasciatus* (n=768), *Cx. vishnui* (n=118), *Cx. tritaeniorhynchus* (n=233), *Cx. gelidus* (n=6), and *An. vagus* (n=79) show in Table 2 and Figure 2.

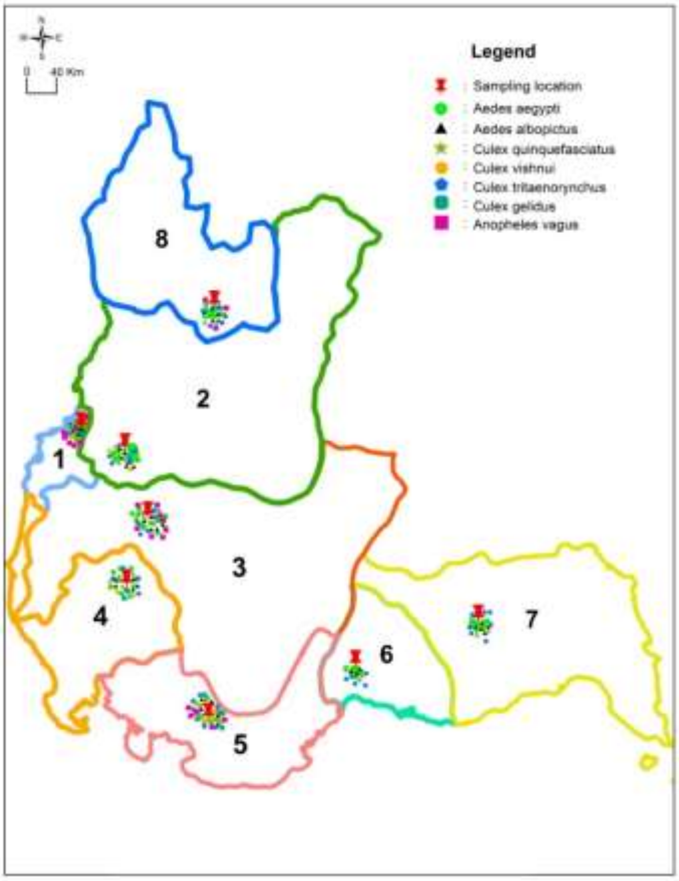

Figure 2. Map of distribution larvae in the inland area of South Sulawesi

The species distribution and numbers of mosquito larvae collected from breeding sites in inland areas of South Sulawesi of mosquito are shown in Table 2 and Figure 2. The larvae breeding habitats preference shows variation between genus and species. Table 3 and Figure 3 show four types of containers with high larval percentages, i.e., used buckets (33.3%), used tires (26.4%), water drums (17.2%), and bathtubs (12.2%).

Table 2. Species and numbers of mosquito larvae collected from breeding sites in inland areas of South Sulawesi

Location	***Ae. aegypti***	***Ae. albopictus***	***Cx. quinquefasciatus***	***Cx. vishnui***	***Cx. tritaenorynchus***	***Cx. gelidus***	***An. vagus***	**Total**
Biringkanaya	345	189	176	23	76	0	16	825
Marusu	276	102	122	13	45	0	0	558
Pattalassang	177	48	98	0	33	0	39	395
Palombangkeng	197	72	73	3	28	1	0	374
Bontoramba	110	29	102	78	18	5	21	363
Sinoa	293	52	16	0	8	0	0	369
Rilau Ale	210	11	54	1	21	0	0	297
Balocci	451	37	127	0	4	0	3	622
Total	**2059**	**540**	**768**	**118**	**233**	**6**	**79**	**3803**

Table 3. Types of containers and percentage of mosquito larvae collected in the inland area of South Sulawesi

Type of containers	**Total number of containers**	**Number of Positive Container**	**Larvae Percentage (%)**						
			Ae. aegypti	***Ae. albopictus***	***Cx. quinquefasciatus***	***Cx. vishnui***	***Cx. tritaenorynchus***	***Cx. gelidus***	***An. vagus***
Used tires	543	311	26.4	17.2	8.7	0	0	0	0
Flowerpot	77	36	6.4	2.0	2.5	0	0	0	0
Plastic cups	160	26	2.2	0	0.1	0	0	0	0
Used Bucket	381	128	10.7	5.9	11.6	0	0	0	0
Used Pans	48	16	5.0	10.7	2.0	0	0	0	0
Used Gallons	33	12	4.7	0	0	0	0	0	0
Trash can	20	9	3.7	4.8	7.3	0	0	0	0
Petals	10	6	0.5	3.3	0	0	0	0	0
Water hole	8	3	0	0	0.4	0	16.0	0	7.3
Irrigation Channel	30	9	0	0	22.1	8.6	12.0	0	13.0
Rice field	10	5	0	0	11.2	22.0	8.0	32.8	79.7
Swamp	8	6	0	0	18.9	54.0	64.0	67.2	0

Water drums	376	289	14.0	16.3	4.0	0	0	0	0
Used cans	63	34	8.6	3.9	1.0	0	0	0	0
Styrofoam	87	17	2.3	1.7	0	0	0	0	0
Pool	7	3	0.6	0.9	0	15.4	0	0	0
Used dipper	23	3	0.4	0.4	0	0	0	0	0
Bath	293	137	7.4	12.2	0	0	0	0	0
Soil pitcher	56	15	1.4	0	0	0	0	0	0
Dispenser	47	20	2.6	0	0	0	0	0	0
Tree hole	28	7	0	16.4	0	0	0	0	0
Crock	12	3	3.0	4.3	10.2	0	0	0	0
Total	**2320**	**1095**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Figure 4. Mosquito breeding places: a. used tires, b. water drum, c. used bucket, d. bath, e. irrigation channel, f. water hole, g. swamp, h. ricefield

The physical properties of water influence the breeding, survival, and adaptation of various organisms, including mosquito larvae. Mosquitoes can breed in various aquatic environments such as in abandoned containers (used tires, water drums and buckets), natural containers (water holes and rock pools) and water bodies with zero salinity (irrigation channels and rice fields). Mosquito larvae require water temperatures ranging from 24.5°C to 34.2°C, water pH ranging from 6.3 to 7.5, and humidity ranging from 67.8 to 89.0 (Table 4).

Table 4. Physical properties of mosquito larvae habitat in the inland area of South Sulawesi

Location	**pH**	**Salinity (ppt)**	**Temperature (°C)**	**Humidity (%)**
Biringkanaya	±6.7-7.2	0	±27.3-32.4	±70.3-85.0
Marusu	±6.3-7.0	0	±24.5-33.2	±69.8-82.8
Pattalassang	±6.9-7.5	0	±26.9-32.0	±73.2-84.2
Palombangkeng	±7.0-7.3	0	±25.3-31.4	±70.5-86.4
Bontoramba	±6.8-7.4	0	±28.0-34.2	±67.8-89.0
Sinoa	±6.6-7.1	0	±28.5-32.5	±70.8-82.4
Rilau Ale	±7.0-7.4	0	±26.2-31.0	±71.0-88.2
Balocci	±7.0-7.2	0	±28.2-34.2	±69.7-86.4

Chi-Square analysis showed significant habitat characteristics with an abundance of *Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus, Cx. vishnui, Cx. tritaeniorhynchus*, while in *Cx. gelidus* and *An. vagus* was not significantly associated (*P≥0.05*). The number of mosquito larvae found was related to pH, salinity, temperature, and humidity (Table 5).

Table 5. Habitat characteristics correlated with the abundance of the mosquito larvae in inland area

Spesies	**Chi-Square Tests**			
		Value	**Df**	**Sig.**
Ae. aegypti	Pearson Chi-Square	16.336	20	0.023*
	Likelihood Ratio	17.602	20	0.044
	Linear-by-Linear Association	0.489	1	0.184
Ae. albopictus	Pearson Chi-Square	13.080	20	0.034*
	Likelihood Ratio	15.134	20	0.514
	Linear-by-Linear Association	0.889	1	0.346
Cx. quinquefasciatus	Pearson Chi-Square	13.753	20	0.019*
	Likelihood Ratio	15.079	20	0.418
	Linear-by-Linear Association	1.631	1	0.202
Cx. vishnui	Pearson Chi-Square	21.342	20	0.031*
	Likelihood Ratio	26.326	20	0.121
	Linear-by-Linear Association	0.845	1	0.158
Cx. tritaenorynchus	Pearson Chi-Square	14.425	20	0.025*

	Likelihood Ratio	15.259	20	0.006
	Linear-by-Linear Association	2.221	1	0.136
Cx. gelidus	Pearson Chi-Square	10.753	20	0.932
	Likelihood Ratio	12.014	20	0.885
	Linear-by-Linear Association	1.055	1	0.304
An. vagus	Pearson Chi-Square	18.525	20	0.488
	Likelihood Ratio	19.618	20	0.418
	Linear-by-Linear Association	0.093	1	0.760

Note: *Correlation is significant at the 0.05 level (2-tailed)

Most of the vectors of arboviruses in humans from the Culicidae family belong to the phylum Arthropoda. Arboviruses are found in tropical and sub-tropical countries, including Indonesia (Myint et al. 2014). Arbovirus infections have made up 30% of all infectious diseases (Jones et al. 2008). *Aedes* sp. lives in tropical and subtropical areas, breeds in plastic containers, tires, pots, leaf petals and can adapt to various environments (Ferede et al. 2018). *Ae. aegypti* and *Ae. albopictus* are vectors of dengue virus (Guo et al. 2016; Ibáñez-Justicia et al. 2020; Satoto et al. 2014).

Breeding places for *Culex* sp. were found in puddles of water, rice fields, irrigation canals, used tires, ditches, soil holes, and unused ponds (Nchoutpouen et al. 2019; Shaman 2010). The abundance of *Culex* sp. mosquitoes has the potential to pose a risk of spreading Japanese encephalitis (Lindahl et al. 2012), Zika (Huang et al. 2016), Usutu, and West Nile viruses (Fros et al. 2015). In addition, *Culex* sp. that is a carrier of the West Nile virus include *Cx. tritaeniorhynchus*, *Cx. pipiens*, *Cx. nigrapalpus*, *Cx. quinquefasciatus*, *Cx. restuans*, and *Cx. modestus* (Golding et al. 2012).

Anopheles sp. is a carrier of malaria that causes the O'nyong-nyong virus in humans (Rezza et al. 2017). Currently, malaria is still a serious health problem in Indonesia. The high risk of death occurs in infants, children under five years, and pregnant women (Jiero & Pasaribu 2021; Patriani et al. 2019). *Anopheles* habitats were found in larger water bodies such as lakes, river banks, swamps, ponds, rice fields, and rivers (Hamidian 2011). The early discovery of breeding sites for arbovirus-carrying mosquito larvae is one of the strategies to prevent the spread of arboviruses.

Distribution of larvae of *Ae. aegypti* in this study was found in Balocci, *Ae. albopictus*, *Cx. tritaenorynchus* and *Cx. quinquefasciatus* in Biringkanaya, *Cx. vishnui* and *Cx. gelidus* in Bontoramba, and *An. vagus* in Pattalassang. The abundance of mosquito larvae in the inland area of each site is related to the proximity of larval habitat containers from residential areas. Based on Augustina et al. (2021), mosquito larvae are found to breed mostly in old tires, puddles, plastic containers, pots, and drains in inland areas. Vector abundance is related to residential areas (Rahma et al. 2020). Characteristics of mosquito larvae breeding sites in the inland areas were found in various containers. *Ae. aegypti* and *Ae. albopictus* was found highest in used tires, *Cx. quinquefasciatus* in irrigation canals, *Cx. vishnui*, *Cx. tritaenorynchus*, and *Cx. gelidus* in swamps, as well as *An. vagus* in the rice fields.

Larvae of *Ae. aegypti* and *Ae. albopictus* is often found in used tires because people store used tires in their yards. People use old tires as artificial pots and waste storage. The disadvantages of using used tires is that they collect rain water, and when neglected, *Ae. aegypti* and *Ae. albopictus* tend to lay eggs in these tires. A study by Ferede et al. (2018) have shown a significant percentage (57.5%) of *Ae. aegypti* breeding in used tires. Other studies have shown a substantial percentage of mix breeding of *Ae. albopictus* and *Ae. aegypti* in used tires ie; 45% (Futami et al. 2020) 26.5% (Higa et al. 2010) and 9.1% (Ratnasari et al. 2021).

Larvae of *Cx. quinquefasciatus* is commonly found in irrigation canals filled with garbage. They Mosquitoes will lay their eggs in slow-flowing, calm, and litter-filled water. *Cx. quinquefasciatus* is widespread in urban, suburban, rural, and remote areas. These mosquitoes are often found in various habitats and are anthropophilic (Muturi et al. 2007; Reuben 1992). The distribution of *Cx. quinquefasciatus* is found in various countries such as Thailand (Kitvatanachai et al. 2005), Singapore (Lam-Phua et al. 2019), Philippines (Carvajal et al. 2018), Vietnam (Ha et al. 2021) and Malaysia (Low et al. 2012; Ng et al. 2016).

Larvae of *Cx. vishnui*, *Cx. tritaenorynchus*, and *Cx. gelidus* were found in irrigation canals, rice fields, and most larvae were found in swamps. Larvae of *Cx. vishnui* and *Cx. gelidus* can be found in lowland and mountainous areas. Habitat characteristics of these two species are found in rice fields, cultivated areas, and livestock. The lowest percentage of *Cx. gelidus* larvae in this study were influenced by residents who did not own livestock such as pigs. *Cx. gelidus* prefer to suck the blood of animals such as pigs and human blood (Lindahl et al. 2012).

The percentage of larvae of *An. vagus* was found in water holes and irrigation channels. The highest number larvae of *An. vagus* was found in rice fields. Some of the villagers are exposed to *An. vagus* because they have rice fields near their houses which support this mosquito to breed. A study by Wharton et al. (1963) found that breeding places for Anopheles were located in swamps. In addition, several potential places include rice fields, waterways, buffalo puddles, and rivers (Maretasari et al. 2019).

The physical factor of water is very influential in the success of breeding and survival of mosquito larvae. These factors can affect the density and diversity of mosquitoes. Water quality and characteristics are determinants of species diversity and mosquito composition (Bashar et al. 2016). This study showed that the water in the container had varying pH, temperature, humidity, and salinity, which could affect the survival of the larvae. A total of eight locations showed that pH, temperature, and humidity supported the breeding of *Aedes*, *Culex*, and *Anopheles*. According to Lubinda et al. (2019) and Chandrasegaran et al. (2020), the larvae of *Ae. aegypti* and *Ae. albopictus* can live at temperatures between -6 and 31.5°C and pH ranging from 4.2 to 9.8. According to Clark et al. (2004), the pH of water can affect the success of mosquito larvae breeding, and mosquito larvae will die at a pH of less than three and more than 12, salinity of less than 0.01 to 6.3 ppt (Medeiros et al. 2020), and humidity of 60-90% (Clements 1992).

This study observes four species of the genus *Culex*, i.e., *Cx. quinquefasciatus*, *Cx. vishnui*, *Cx. tritaenorynchus*, and *Cx. gelidus*. In general, *Culex* can survive at temperatures from 24°C to 29°C. However, survival will decrease at low temperatures (≤12°C), at high temperatures (≥32°C), and die at 35°C (Grech et al. 2013). The larvae prefer slightly alkaline water (pH<8), 60% to 98% humidity (Muturi et al. 2009), and the highest salinity tolerance of 6 ppt (Fakhriedzwan et al. 2011).

The *Anopheles* larvae found in this study was *An. vagus*, which lives in rice fields. The pH value in Biringkanaya, Pattalassang, Bontoramba, and Balocci is 6.7 to 7.58. According to Maretasari et al. (2019), the pH value of rice fields is 6-8. Akhiriyanti and Nugroho (2019) reported that mosquito larvae prefer a pH range of 6.8-7.2. The temperature ranged from 26.9°C to 34.2°C in the fields where *An. vagus* larvae were discovered. According to Novianto et al. (2021) and Kengluecha et al. (2005), larvae of *An. vagus* can live in water temperatures ranging from 25.4°C to 32°C. Rice fields are areas without shade so that sunlight can penetrate the water and provide a higher temperature value than areas shaded by trees. Warm water due to sunlight may be a determining factor for larval development and accelerate the growth of *Anopheles* sp. In addition, warm temperatures allow more microorganisms as a food source for mosquito larvae (Minakawa et al. 1999).

The density of larvae has a significant effect on their breeding sites, such as *Ae. aegypti*, *Ae. albopictu*s, *Cx. quinquefasciatus*, *Cx. visnui*, and *Cx. tritaenorynchus*. The density of mosquito larvae correlates with the conditions of their breeding sites (Medeiros et al. 2020; Vanlalruia et al. 2014). According to Madzlan et al. (2016), in addition to the factors of breeding sites, the physical condition of the water influences the density of mosquito larvae. Due to limited data on larval breeding sites and number of larvae found, the correlation cannot be determined for *Cx. gelidus* and *An. vagus* in this study.

Several studies have found that the survival of this species *Culex* sp. is linked to the habitat's environmental conditions, predators, pH, rainfall, soil conditions, aquatic plants, and sunlight intensity (Klinkenberg et al. 2003; Mutero et al. 2000; Mwangangi et al. 2006). Another factor to consider is the turbidity of the water, which influences mosquitoes' breeding attractiveness. Insoluble soil particles, organic matter, microorganisms, and other materials contribute to water turbidity (Kean et al. 2015; Munga et al. 2005). Mosquitoes are a formidable disease vector because they are found worldwide and adapt to various environmental conditions (Chandrasegaran et al. 2020). Forests, mountains, plains, deserts, tropical forests, salt marshes, and tidal zones are among the habitats where the species can breed (Foster & Walker 2019). This study concludes that mosquitoes can breed in various types of neglected water containers. Therefore, public awareness of mosquito larvae ecology in the environment is essential for reducing the density and intensity of vectors as arbovirus carriers in humans.

CONCLUSION

The application of vector control methods generally focused on the environment of urban areas. The availability of mosquito larvae in remote areas could have health consequences through neglected arbovirus and parasite disease incidence. Therefore, the results of this study are expected to be input into the implementation of policies related to vector control which are not only based on the location where DHF patients are found but also consider vector findings based on larvae surveys found in remote areas.

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AUTHORS DECLARATIONS

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Conflict of Interest
The authors declare that there's no conflict of interests.

Ethics Declarations
This research did approve by the Health Research Ethics Committee of the Hasanuddin University Medical Faculty with the attached number 558/UN4.6.4.5.31/PP36/2020

Data Availability Statement
My manuscript has no associated data

Authors' Contributions
AR, IW and ARJ conceived this research and designed experiments; HA, DM and MI participated in the design and interpretation of the data; AR, MP, AIT, A and H performed experiments and analysis; AR, IW, S and ARJ wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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