Research Article

Physicochemical Characters of Mosquitoes Natural Breeding Habitats: First Record in High Dengue Hemorrhagic Fever Cases Area, East Java, Indonesia

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ABSTRACT
This research aims to identify physicochemical characteristics in natural breeding habitats/ phytotelmata of dengue vector—including *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus*. The research was conducted during rainy season and pre-dry season (from January to June 2017) in the region with the high cases of Dengue Hemorrhagic Fever (DHF). The entomological survey was carried out by stratified random sampling in urban and rural areas in order to find potential breeding habitats, every natural breeding habitats in sampling location were checked for the presence of *Aedes* larvae. Physicochemical characters that consist of temperature, turbidity, carbon dioxide, ammonia, nitrate, sulphate, pH and dissolved oxygen were recorded. Larval species were taken and then identifying activities were conducted in the Laboratory of Entomology. Data were analysed using the Chi-square test. Results showed that only dissolved oxygen that significantly associated with larval abundance (p=0.039). while others are not significantly associated. Whereas, other characters are associated with each other, carbon dioxide associated with the ammonia and sulphate (p=0.001; p=0.028). Turbidity associated with the dissolved oxygen (p=0.022) and pH associated with nitrate (p=0.001).

Keywords: *Aedes aegypti*, *Aedes albopictus*, dengue, East Java, natural breeding habitat, phytotelmata

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a public health problem in tropical and sub-tropical countries. World Health Organization (WHO) estimated that 50 million people around the world were infected, half of whom need hospitalization. The highest proportion of DHF patients is children with the age of less than five years old (WHO SEARO, 2011). DHF is caused by dengue virus that transmitted by mosquito bites so that its classified as mosquito-borne diseases. DENV has four serotypes namely DENV-1, DENV-2, DENV-3 and DENV-4 (Gubler, 2002). *Aedes (Stegomyia) aegypti* is known as a primary vector of DHF, while *Aedes (Stegomyia) albopictus* is a secondary vector in South East Asia and Western Pacific (World Health Organization, 2008).

East Java Province is one of the endemic provinces that still have high cases in DHF. Based on the data from (Ministry of Health Republic of Indonesia, 2018), the number of DHF cases in East Java Province in 2016 was 24005 cases, with the incidence rate of 61.43 every 100.000 people. DHF prevention methods have been implemented by East Java government such as health promotion, surveillance, periodically larval controlling, public health behavior (drain, buried and close also called 3M) (Ministry of Health Republic of Indonesia, 2018).

Transmission of DHF depends on season

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conditions and availability of its vector which is very sensitive to temperature and moisture (Thai and Anders, 2011). Dengue vaccine still on the developing phase prior to finding high efficacy against all serotypes (Wilder-Smith *et al.*, 2016). Hence, the most effective controlling program of DHF is vector control.

Understanding bionomic and characteristic of vector is crucial in controlling the program. Distribution of *Aedes aegypti* and *Aedes albopictus* is strongly correlated with the global climate (Ravikumar *et al.*, 2013). In case, the developmental cycle of *Aedes aegypti* depends on some environmental factors like rainfall, temperature, relative humidity (Eisen *et al.*, 2014).

Aedes mosquitoes spread widely and have two breeding habitats, indoor and outdoor. Meanwhile, based on their formation, breeding habitats of *Aedes* mosquitoes are divided into two kinds, that are natural and artificial breeding habitats. Artificial breeding habitat is a breeding habitat formed because of human/animal activity such as tank, bathtub, crock, vast, tank, cans and tyres. Natural breeding habitats is a breeding habitat formed without human/animal activity such as pool, tree hole, leaf axils, fruit shells, fallen leaf, fallen spathe and so on (Ali *et al.*, 2014; Chadee *et al.*, 1998).

Research on natural breeding habitats is still limited. Some countries, such as USA and Africa, have been doing research on potential natural breeding sites that might be an account of DHF vector, such as rock holes, tree hole, leaf axils, bamboo joints, coconut shells, and also Bromelia (Chadee *et al.*, 1998). Some Bromeliad species become mosquitoes breeding habitats (Shultis,

2009). Research from (Simard *et al.*, 2006) showed that the highest natural breeding site in Cameroon Africa is a tree hole. Based on this problem, they did research on various kinds of phytotelmata that were found in areas with high DHF cases in East Java Province, Indonesia.

Although intensively control program was conducted, only one research on natural breeding site in Indonesia already published in the past ten years (Rosa *et al.*, 2017). Based on this understanding, this research aims to identify the physicochemical characters that support growth and development of Aedes larvae, specifically in natural breeding habitats.

MATERIALS AND METHODS

Materials
The instruments used in this research were vial bottles (100 mL and 10 mL), tray, plastic pipette, universal pH paper (Merck & Co, NewYork), thermometer (Thermo, Indonesia), DO meter (Lutron DO meter 5510), sling psychrometer TS90, object-glass, cover glass, labels, and plastic glass, gauze, rubber, identification key using larval and adult identification key of *Aedes* in Java, Indonesia.

Methods
The entomological survey was conducted during rainy season and pre-dry season (January to June 2017) in an area that represented as high dengue risk in East Java Province that is Pacitan, Sidoarjo, Malang, Jombang, Banyuwangi, and Bangkalan. Study areas were shown in Figure 1 with legend (A: Pacitan Residence; B: Malang Residence; C:

Figure 1. Research area of entomological survey were signed by red circle (EpiInfo, 2019).

Banyuwangi Residence; D: Sidoarjo Residence; E: Bangkalan Residence; F: Jombang Residence). Study areas were chosen based on unpublished data from East Java Province Health Office, with detail on number of population and number of cases below (Table 1).

Table 1. Number of population and number of cases in each study area.

Legend	Study Area	Population Number	Number of DHF cases
A.	Pacitan	552.307	1149
B.	Malang	2.560.675	950
C.	Banyuwangi	1.599.811	1319
D.	Sidoarjo	2.150.482	1455
E.	Bangkalan	962.771	801
F.	Jombang	1.247.303	741

Based on (Kitching, 1971) phytotelmata can be categorized as six groups namely leaf axils, tree holes, tree stumps, fallen spathe, fruit shells and fallen leaf. Every phytotelma found during research was checked by using a flashlight in order to confirm the presence of *Aedes* larvae. The simple way to differentiate between *Aedes* larvae and other larvae was by checking their siphon and their movement pattern. A total of 53 water samples were collected from various phytotelmata (nine phytotelmata from study site A, five from B, ten from C, fourteen from D, seven from E, and seven from F). By using plastic pipettes, every larva found was collected into 10 mL vial bottle, while 100 mL water sample was added into vial bottle. Every sample was labeled based on the date of collection, location of phytotelmata, phytotelma categories and the name of species. Water samples then packed in cold boxes and were transported to the laboratory for further analysis. Some parameters were measured directly on the spot, such as temperature, dissolved oxygen, and pH using thermometer (Thermo, Indonesia), Lutron DO meter 5510, and universal pH paper (Merck, New York) whereas other parameters measurement were done by analysis service in Balai Besar Teknik Kesehatan Lingkungan Surabaya. Another physicochemical factors (turbidity, carbon dioxide, ammonia, sulphate, and nitrate) were analyse used spectrophotometric based on the guideline of American Public Health Association (American Public Health Association, 1998) In order to identify the species of larvae, fourth instar stage of larvae were killed by 4% formalin, while pupae were maintained until they become adult mosquitoes. Data were analyzed using SPSS for Chi-square test, with the normalized test was done

before.

RESULTS AND DISCUSSION

Distribution of phytotelma and physicochemical factors in each study area

Distribution of each phytotelma that contains *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* larvae was shown in Table 2. The number of *Aedes* larvae counted on phytotelmata and physical parameters were shown in Graphic 1. Bivariate correlations between physicochemical factors and larval abundance were shown in Table 3.
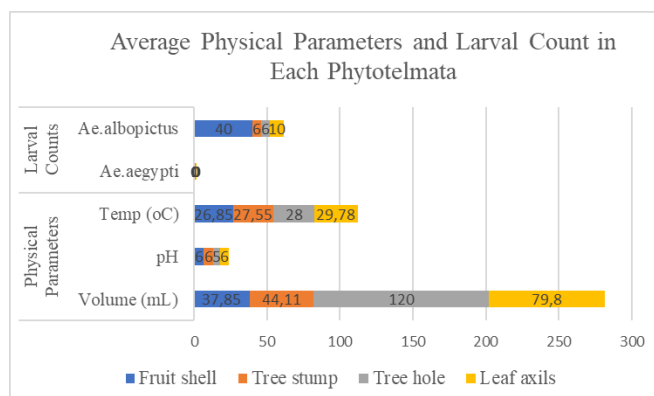
Figures of phytotelmata

There were 4 types of phytotelmata found in this research namely leaf axils, tree stumps, fruit shells, and tree holes (Figure 2).

Leaf axils of *Neoregelia* 'hybrid'

Tree stumps of *Bambusa balcooa*

Tree hole of *Hibiscus macrophyllus*

Fruit shells of *Cocos nucifera*

Figure 2. Different types of phytotelmata that were found.

Physical Parameters and Larval Counts in each kind of phytotelma

Graphic 1. Average physical parameters and larval count in each phytotelma.

Table 2. List of phytotelmata that were found during entomological survey.

No.	Plant Species	Plant Family	Leaf Axils	Tree Stumps	Fruit Shells	Tree Holes
1.	*Neoregelia carolinae*	Bromeliaceae	√			
2.	*Neoregelia* 'Royal Burgundy'	Bromeliaceae	√			
3.	*Musa paradisiaca*	Musaceae	√			
4.	*Alcantarea imperialis*	Bromeliaceae	√			
5.	*Dracaena fragrans*	Asparagaceae	√			
6.	*Bambusa balcooa*	Poaceae		√		
7.	*Colocasia esculenta*	Araceae	√			
8.	*Hibiscus macrophyllus*	Malvaceae				√
9.	*Xanthosoma sagittifolium*	Araceae	√			
10.	*Guzmania lingulate*	Bromeliaceae	√			
11.	*Portea petropolitana*	Bromeliaceae	√			
12.	*Agave Americana var.Marginata*	Bromeliaceae	√			
13.	*Neoregelia hybrid*	Bromeliaceae	√			
14.	*Cocos nucifera*	Arecaceae			√	

Table 3. Bivariate Correlations between Physicochemical Factors and Larval Abundance.

	Temperature	Turbidity	Carbon dioxide	Ammonia	Nitrate	Sulphate	pH	DO
Temperature		0,763	0,787	0,853	0,367	0,321	0,474	0,225
Turbidity	0,763		0,908	0,755	0,493	0,234	0,978	0,022*
Carbon dioxide	0,787	0,908		0,001*	0,092	0,028*	0,779	0,556
Ammonia	0,853	0,755	0,001*	-	0	0	0,196	0,399
Nitrate	0,367	0,493	0,092	0	-	0	0,001*	0,371
Sulphate	0,321	0,234	0,028*	0	0	-	0	0,281
pH	0,474	0,978	0,779	0,196	0,001*	0	-	0,996
DO	0,225	0,022*	0,556	0,399	0,371	0,281	0,996	
Larval abundance	0,054	0,977	0,38	0,874	0,601	0,545	0,628	0,039*

Discussion

The occurrence of mosquito larval in natural breeding sites/ phytotelmata related to the oviposition site selection. The mosquito activity can be influenced by physicochemical factors that can play as an attractant or a deterrent (Thangamathi *et al.*, 2014). Rainfall, relative humidity, temperature, and wind speed are known as environmental factors that can affect mosquitoes laying eggs, whereas visual, olfactory and tactile responses are biological factors related to metabolism in mosquito's body. Current research found four kinds of phytotelmata, which are leaf axils, tree stumps, fruit shells and tree holes. Phytotelmata in this research were classified into 7 families, which are family of Bromeliaceae, Musaceae, Asparagaceae, Poaceae, Araceae, Malvaceae, and Arecaceae. Leaf axils were the most breeding site that was found followed by tree stumps, fruit shells, and tree holes.

A total of 714 larvae were collected, consisting of 691 larvae *Aedes (Stegomyia) albopictus* and 23 larvae *Aedes (Stegomyia) aegypti*. Some water parameters are important in public health and used as an indicator of drinking-quality (World Health Organization, 2008). Studying physical and chemical factors is significant in order to understand the bionomics of *Aedes*. Some studies found that physicochemical factors are associated significantly with the larval species. (Ghanbari *et al.*, 2019) mentioned that 7 physicochemical factors correlate to *Anopheles* species namely pH, total hardness, nitrate, phosphate, calcium, electrical conductivity, and sulphate. This research found that the temperature range of larval breeding sites is 27-32° C; suitable with (Hanafi-Bojd *et al.*, 2012) result shows that the temperature of breeding sites during larval collection is 20-32°C. Temperature is a crucial factor that can affect the growth, development, and survival of mosquito. The more increase in temperature, the more decrease in mosquito body size (Rueda *et al.*, 1990).

Leaf axils as the most frequent kind of breeding site in this area consisting of many species based on the bromeliad identification key by (Derek Butcher and Dean Fairchild, 2017) namely *Neoregelia carolinae, Neoregelia* 'Royal Burgundy', *Musa paradisiaca, Alcantarea imperialis, Dracaena fragrans, Colocasia esculenta, Xanthosoma sagittifolium, Guzmania lingulata, Portea petropolitana, Agave Americana* var. Marginata, and *Neoregelia hybrid*. Tree stumps consist of two species that is *Bambusa balcooa* and *Bambusa vulgaris*. Otherwise, tree holes consist of *Hibiscus macrophyllus*. Bad management of rubbish especially on fruit shells advances to the occurrence of larval breeding sites, especially the fruit shells of *Cocos nucifera*. This finding corresponded to the research of (Thangamathi *et al.*, 2014). In contrast, larval counts on *Cocos nucifera* were mostly *Aedes (Stegomyia) albopictus*, which was different from the result of (Thangamathi *et al.*, 2014) *Cocos nucifera* is mostly found on shady area which can provide ideal condition for mosquito's oviposition.

Reports of breeding site especially natural breeding sites in Indonesia is still limited, only one article that has been published (Rosa *et al.*, 2017). Meanwhile, thus research showed more various kinds of phytotelmata: tree holes, tree stumps, fallen spathe, fruit shells, and fallen leaves. However, *Aedes* species found are different from current research that are *Aedes (Stegomyia) albopictus* and *Aedes (Stegomyia) crysolineatus*. The turbidity value in current research is in a range between 0.04-2.42 NTU. The highest turbidity was found in Pacitan, while the lowest was found in Bangkalan. However, there was still no exact values of physicochemical factors related to the abundance of larval mosquito-like both mentioned by Ghanbari *et al.* (2019) and (Hanafi-Bojd *et al.*, 2012). Turbidity is one of the physical factors that have little difference in both *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia)* albopictus. Turbidity value related to the accumulation of eroded soils after rain or other materials from stagnant water. A study shows that turbid water attracts more female *Anopheles*, but at some points will affect mortality (Mwangangi *et al.*, 2010; Sehgal & Pillai, 1962). However, some studies reveal that turbid water has an importance which provides a suitable place for the development of first till third larval instar and pupal stage. Other than that, turbid water can suppress the development of the fourth larval instar (Sehgal & Pillai, 1962). This controversial still need to be supported with massive research, so that vector control program related to the turbidity of water can be conduct based on currently finding.

Dissolved oxygen shows a significant association with the larval counts. The range value of dissolved oxygen in this research was between 3.4 and 5.7 ppm. Dissolved oxygen contained in water related with the carbon dioxide tension. Association between physicochemical factors and larval abundance was proven. Variable that shows a positive association with the larval abundance in this research was dissolved oxygen (p=0.039). These results are in agreement with other research reported (Muturi *et al.*, 2008). Dissolved oxygen is an important factor that affected survival and mosquito breeding site (Oyewole *et al.*, 2009)

Another physicochemical factor shows negative correlation with the larval abundance: temperature (p=0.054); turbidity (p=0.977); carbon dioxide (p=0.38); ammonia (p=0.874); nitrate

(p=0.601); sulphate (p=0.545); pH (p=0.628). Chi-square test was used in the analysis, and the result was shown in Table 3. Whereas, characters are associated with each other, carbon dioxide associated with the ammonia and sulphate (p=0.001; p=0.028). Turbidity associated with the dissolved oxygen (p=0.022) and pH associated with nitrate (p=0.001). Our findings show that temperature didn't have significant association (p=0.054) with the larval abundance. Even though, some studies reveal the influence of temperature on the abundance of mosquito larvae, regarding the classification of mosquitoes as a poikilothermic animal. The more increase in water temperature, the faster mosquito larval will develop. Other than that, adult size of mosquito will decrease, and at the highest temperature, mortality will be increased (Bayoh and Lindsay, 2003; White, 1974). Interaction between temperature, pH, and ammonia can give various impacts. Ideal pH for mosquitoes ranged from 8 to 8.8, high pH above the range will disturb the survival of mosquito related with the increase of ammonia (Kwasi *et al*., 2012).

Bivariate correlation between nitrate and pH value shows a significant association (p=0.001). Mosquito larval were known can tolerate various level of nitrate, and nitrate value related with other organism activities. A study reported that increasing nitrate can increase the density of mosquito larvae (Sunish and Reuben, 2002). Meanwhile, there is also a report that shows decreasing nitrogen can affect increasing of larval densities (Mala and Irungu, 2011).

Water volume that holds in every phytotelma was also measured as additional parameters. Then, it was classified into each kind of phytotelma. Based on Graphic 1, tree hole is a kind of breeding site that can hold high water volume (120 mL), followed by leaf axils (79.8 mL), water-hold in tree stumps and fruit shell does not differ significantly that is 44.11 and 37.85 mL respectively. Stratified random sampling in urban and rural area tributes to the several breeding sites that were found. Bromeliads are mostly found in urban area, whereas the majority of breeding sites in this research in rural area was fruit shells. Each of breeding sites consists of various stages of larval; some of them have been in pupal stage. It can be an indicator that breeding site is suitable for the development of larval, regarding to the optimum value of every physicochemical factor.

Current investigations found some containers contain both larval of *Aedes aegypti* and *Aedes albopictus* in one container. Survivorship of both species in one container cannot be separated from interspecific competition supported by environmental factors. Nutritional sources for mosquito larvae come from physicochemical factors, even though in excessive ratio will give a negative impact. Sulfate is a natural matter that composed of sulfur and oxygen, as a result of plant and animal that decayed. Water in phytotelmata contained sulphate may be due to the position under canopy. The higher sulphate, the more larval abundance will increase (Liu *et al*., 2012). Physical variables can take a role in this case and directly affect the competitive interactions, but how the mechanism and prevalence of it are still unclear (Costanzo *et al*., 2005; Dunson and Travis, 1991). Some studies show that variety of environmental factors affect the spread of *Aedes albopictus* (Alto and Juliano, 2009). Detritus as a well-known factor affects competition between both species when holding in one container (Yee *et al*., 2007). Different types of detritus lead to creating different quantities of microorganisms (Yee *et al*., 2007) that can provide a difference in both quantity and quality of food available for larvae.

Our observation shows that water appearance in each breeding site is different; some breeding sites consist of many detritus in the form of fall leaves, while another does not contain it. Both *Aedes aegypti* and *Aedes albopictus* are vectors of mosquito-borne diseases such as yellow fever, dengue, and West Nile Virus, so it makes an interesting thing when basic bionomics of them are understood (Yuill, 1986). Therefore, research about physicochemical characters, especially under laboratory conditions, still needs to be conducted, regarding the different geographical area can lead to different environmental conditions.

CONCLUSION
Dissolved oxygen shows significantly associated with larval abundance (p=0.039) while others are not significantly associated. Whereas, other characters are associated with each other, carbon dioxide associated with the ammonia and sulphate (p=0.001; p=0.028). Turbidity associated with the dissolved oxygen (p=0.022) and pH associated with nitrate (p=0.001). Research about physicochemical characters especially under laboratory conditions still needs to be conducted, regarding the different geographical area can lead to different environmental conditions and useful for making integrated vector management policy.

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