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Isna Lailatur Rohmah
Entomology Laboratory, Division of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia

Upik Kesumawati Hadi
Entomology Laboratory, Division of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia

Susi Soviana
Entomology Laboratory, Division of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia

Correspondence
Upik Kesumawati Hadi
Entomology Laboratory, Division of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia

Larval breeding habitat of *Simulium* (Diptera: Simuliidae) around stream of waterfall areas of Bogor forest management unit

Isna Lailatur Rohmah, Upik Kesumawati Hadi and Susi Soviana

Abstract
The aim of this study was to identify the diversity of *Simulium* in 19 waterfalls of Bogor Forest Management Unit, as well as to know the characteristics of black fly larval stage breeding habitats that affect the abundance of *Simulium* population. Preparation samples were performed manually from September-December 2017, along with *Simulium* larvae habitat characteristics measurements simultaneously. The most common *Simulium* species found in 19 waterfalls of Bogor Forest Management Unit were *Simulium* (*S.*) *upikae* (36.31%), followed by *Simulium* (*S.*) *argyrocinctum* (36.11%), *Simulium* (*S.*) *eximium* (11.12%), and *Simulium* (*S.*) *celsum* (11.04%). Of the 19 waterfalls of Bogor Forest Management Unit observed, the highest number of collections of larvae and pupae found in Panjang Waterfall. Pearson correlation analysis showed significant relationship between water temperature, stream width, velocity, the canopy cover with the amount of *Simulium* found.

Keywords: Blackfly, Bogor forest management unit, habitat, larval, waterfall

1. Introduction

Simuliidae (black fly) is a group of biting flies which was important as blood-sucking flies and have the ability to transmit pathogens. The only genus of Simuliidae found in Indonesia was *Simulium* [1]. The black fly was well-known as a vector of myxomatosis, Trypanosomiasis in bird, and Venezuelan Equine Encephalitis [2]. Other disease transmitted by *Simulium* was leucocytozoonosis in bird and onchocerciasis [3-5]. Onchocerciasis was known as a parasitic disease caused by a filarial worm, *Onchocerca volvulus*, that prevalent in Africa and Latin America. The disease was transmitted through the bite of a female *Simulium* from one person to another. The infection caused itching of the skin, lesions in the eye and blindness [6]. It was estimated that 25 million of people in the world suffer from onchocerciasis, and as many as 300 000 people suffered from blindness, 800 000 people experienced visual impairment and 123 million people at risk for this disease [7].

The *Simulium* fly was widely distributed in the world, these flies included into members of a holometabola insect with their prestige stages in aquatic habitat [8]. *Simulium* had a unique attitude in choosing breeding sites, which liked the river that had clear water and heavy flow. These flies lived adjacent to or move along the flow of clear and calm water [9]. Like other aquatic insects, black fly acted as a bioindicator of river environmental quality, this was because aquatic insects including black fly can be used to assess the ecological integrity of rivers [10]. The immature stage of black fly cannot be found in a high-carbon contaminated environment such as industrial areas [11]. The *Simulium* fly habit that liked the swift and non-polluted water flow was very suitable for the water flow conditions in the Bogor Forest Management Unit waterfall. Bogor Forest Management Unit, which managed 19 waterfalls scattered in several areas, as well as a tourist attraction in Bogor, was quite well known and visited.

Research on morpho-taxonomy of black fly in Indonesia had been widely applied [12-16, 1]. A recent review suggested that the number of species of *Simulium* flies in Indonesia was 124 species [1]. Ecological factors affecting the abundance and distribution of the *Simulium* fly were still not widely studied. Based on the description, the natural potential of waterfall areas of Bogor Forest Management Unit as *Simulium* habitat and the lack of information about ecological factors influencing the development of black fly in Indonesia, encourage the need of this research.

The present study aimed to identify the diversity of *Simulium* and to analyze the characteristics of larval habitats that affect the larval population *Simulium* in the streams around the waterfalls of Bogor Forest Management Unit.

2. Material and Methods

Larval collections conducted from September to November 2017 in 19 waterfalls streams. The collection of larvae and pupae was done at 10 different points in the river flow around Bogor Forest Management Unit waterfalls which located at of 483-1368 metres above sea level. The 19 waterfalls observed were Putri Kencana Waterfall, Cinta Waterfall, Bujang Waterfall, Leuwi Hejo Waterfall, Barong Waterfall, Cipamingkis Waterfall, Ciherang Waterfall, Arca Waterfall, Cibeet Waterfall, Cisarua Waterfall, Cidulang Waterfall, Cibereum Waterfall, Cilember Waterfall, Panjang Waterfall, Naga Waterfall, Sawer Waterfall, Gede Waterfall, Kembar A Waterfall, and Kembar B Waterfall (Fig 1).

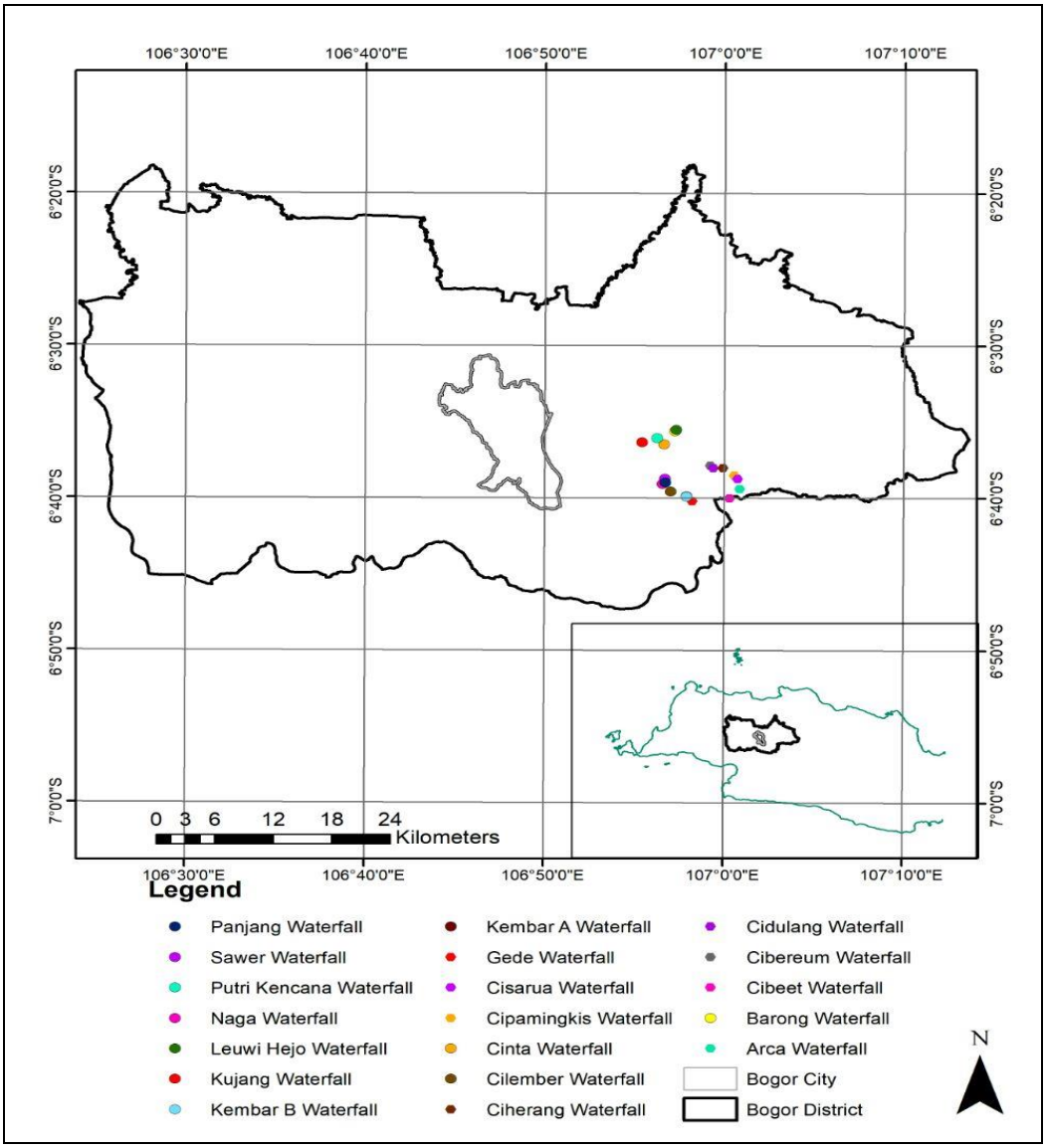

Fig 1: Location of blackflies sampling in the stream around waterfall areas of Bogor Forest Management Unit.

2.1 Collection of immature stage and identification

The collections of larvae and pupae of *Simulium* were carried out on 19 waterfalls of Bogor Forest Management Unit, each waterfall was taken at 10 different points for 15 minutes each point. Larvae and pupae attached to the leaves, stems, roots, and stones contained in the heavy stream were taken with manual tweezers. The larvae found were inserted into a bottle containing 70% alcohol. Meanwhile, the pupae found were inserted into individual bottles, then placed in a plastic container and wrapped in a wet towel to keep the moisture in order to be mature insects. The pupae who transformed to be mature *Simulium* were put into a 70% alcohol bottle. The identification of *Simulium* was conducted at the Medical Entomology Laboratory Faculty of Veterinary Medicine Bogor Agricultural University [14].

2.2 Measurement of immature habitat

The characteristics of immature habitats measured in this study consisted of water temperature (ºC), water pH, streams depth (cm), streams width (m), and velocity (cm/sec), altitude (metres above sea level), canopy cover, riparian vegetation. Temperature and pH were measured using a thermometer and a digital pH meter. Meanwhile, the stream depth and stream width were measured using the meter. The measurement of velocity was conducted using a strapped ball, then the measurement was done by means of the ball being removed and timed using the stopwatch from the time of start releasing the ball until the strap was stretched straight. Measurement of altitude and coordinate points conducted using GPS, while the canopy cover and the riparian vegetation of the stream were measured following the protocol of McCreadie *et al*., (2006) [17].

2.3 Statistical Analysis
The diversity of *Simulium* species found was presented as a descriptive. Meanwhile, the association of habitat characteristics of the immature stage of *Simulium* with the number of larval *Simulium* found was tested using SPSS 17.0 with Pearson correlation test.

3. Results
The present study results showed that of 3 525 *Simulium* larvae found (Table 1) that there were 14 species belonging to 1 genus and 4 subgenera (Table 2). The highest number of larvae was found in Panjang Waterfall (549; Table 1). Meanwhile, most species found to originate from the *Simulium* subgenus (8 species) consist of *Simulium* (*Simulium*) *argyrocinctum*, *Simulium* (*Simulium*) *eximium*, *Simulium* (*Simulium*) *upikae*, *Simulium* (*Simulium*) *nobile*, *Simulium* (*Simulium*) *sigiti*, *Simulium* (*Simulium*) *iridescens*, *Simulium* (*Simulium*) *nebulicola*, *Simulium* (*Simulium*) *celsum*; followed by the subgenus *Gomphostilbia* (4 species) consisting of *Simulium* (*Gomphostilbia*) *sundaicum*, *Simulium* (*Gomphostilbia*) *batoense*, *Simulium* (*Gomphostilbia*) *parahiumum*, *Simulium* (*Gomphostilbia*) *gyorkosae*; and the sub genus of *Nevermania* and *Wallacellum* were one species each, namely *Simulium* (*Nevermania*) *feuerboni* and *Simulium* (*Wallacellum*) sp. (Table 2). Thirteen species were found to have been reported on the island of Java, but there was one newly discovered species of *Simulium* (*Wallacellum*) sp. The number of dominant species at the study sites was *Simulium* (*S.*) *upikae* (36.31%), *Simulium* (*S.*) *argyrocinctum* (36.11%), *Simulium* (*S.*) *eximium* (11.12%), and *Simulium* (*S.*) *celsum* (11.04%). Meanwhile, other species found were <10%. (Table 2).

Waterfalls managed by Bogor Management Unit have a variety characteristics of Simulium habitat. Characteristics of the larval habitat of *Simulium* observed in this study consisted of temperature, pH, stream depth, stream width, velocity, altitude, canopy cover, and riparian vegetation of the stream (Table 3). Based on Pearson correlation analysis, there was significant correlation between water temperature (P-value = 0.036 <α = 0.05), stream width (P-value = 0.004 <α = 0.05), velocity (P-value = 0.007 <α = 0.05), canopy cover (P-value = 0.008 <α = 0.05) with the number of *Simulium* larvae found. Meanwhile, the stream depth, pH, altitude, and riparian vegetation did not show a significant relationship with the number of found *Simulium* larvae (P value> α = 0.05) (Table 4).

Table 1: Description of geographical location and number of *Simulium* found on 19 waterfalls in Bogor Forest Management Unit Area from September-November 2017

Waterfall Name	Coordinate point	Altitude (meters above sea level)	Number of *Simulium*
Putri Kencana Waterfall	S6° 36.097' E106° 56.252'	616	33
Bujang Waterfall	S6° 36.363' E106° 55.433'	559	121
Cinta Waterfall	S6° 36.493' E106° 56.642'	743	8
Leuwi Hejo Waterfall	S6° 35.545' E106° 57.327'	483	29
Barong Waterfall	S6° 35.638' E106° 57.248'	520	52
Cipamingkis Waterfall	S6° 38.535' E107° 00.560'	1028	249
Ciherang Waterfall	S6° 38.051' E106° 59.940'	1061	398
Arca Waterfall	S6° 39.418' E107° 00.857'	1185	121
Cibeet Waterfall	S6° 40.020' E107° 00.297'	1062	63
Cisarua Waterfall	S6° 38.760' E107° 00.746'	1221	316
Cidulang Waterfall	S6° 38.047' E106° 59.394'	1109	62
Cibereum Waterfall	S6° 37.890' E106° 59.244'	1126	50
Cilember Waterfall	S6° 39.593' E106° 57.032'	1020	263
Panjang Waterfall	S6° 38.996' E106° 56.720'	983	549
Naga Waterfall	S6° 39.094' E106° 56.573'	923	247
Sawer Waterfall	S6° 38.751' E106° 56.701'	1034	256
Gede Waterfall	S6° 40.226' E106° 58.231'	1368	26
Kembar A Waterfall	S6° 39.913' E106° 57.900'	1204	292
Kembar B Waterfall	S6° 39.918' E106° 57.906'	1182	390

Table 2: Variety of species of *Simulium and* number of *Simulium* found in 19 waterfalls in Bogor Forest Management Unit, September-November 2017

Species	Number	(%)
Simulium (*Nevermania*) *feuerboni*	1	0.03
Simulium (*Gomphostilbia*) *sundaicum*	10	0.28
Simulium (*Gomphostilbia*) *batoense*	32	0.91
Simulium (*Gomphostilbia*) *parahiyangum*	1	0.03
Simulium (*Gomphostilbia*) *gyorkosae*	1	0.03
Simulium (*Simulium*) *argyrocinctum*	1273	36.11
Simulium (*Simulium*) *eximium*	392	11.12
Simulium (*Simulium*) *upikae*	1280	36.31
Simulium (*Simulium*) *nobile*	1	0.03
Simulium (*Simulium*) *sigiti*	38	1.08
Simulium (*Simulium*) *Iridescens*	10	0.28
Simulium (*Simulium*) *nebulicola*	16	0.45
Simulium (*Simulium*) *celsum*	389	11.04
Simulium (*Wallacellum*) sp.	81	2.30

Table 3: Characteristics of *Simulium* habitat on stream around waterfalls of Bogor Forest Management Unit, September-November 2017

Variable	Minimal value	Maximal value	Average	Standard Deviation
Water temperature (˚C)	18.19	23.17	20.55	1.49
Water pH	4.64	8.14	7.01	1.11
Stream depth (cm)	15.28	93.3	21.25	5.47
Stream width (m)	0.5	9	3.31	2.52
Velocity (cm/sec)	54.81	96.97	78.37	16.82
Altitude (metres above sea level)	483.2	1368.3	969.95	260.96
Canopy cover	Open	Forest		
Riparian vegetation	Open	Complete		

Table 4: Result of Pearson correlation test number of *Simulium* larvae found with the habitat characteristics, September-November 2017

No	Variable	Number of *Simulium* larvae	
		r (Correlation coefficient)	*p-value*
1	Water temperature (˚C)	-0.423	0.036[a]
2	Water pH	0.167	0.247
3	Stream depth (cm)	-0.303	0.103
4	Stream width (m)	-0.411	0.004[a]
5	Velocity (cm/sec)	0.557	0.007[a]
6	Altitude (metres above sea level)	0.302	0.104
7	Canopy cover	0.548	0.008[a]
8	Riparian vegetation	0.029	0.452

Information: Superscript letter in the column shows a significant relationship (P Value <0.05)

4. Discussion

Based on the results of these studies indicate that waterfalls in the area of Bogor Forest Management Unit served as suitable habitats for breeding of the immature stage of *Simulium*. Three sub genera of *Simulium* have been found in Java Island, while the sub genus *Wallacellum* is a new sub-genus found on the Java Island. Previous study by Takaoka and Davies [14] reported that there were only three sub genera of *Simulium* in Java Island, namely sub genus *Simulium*, *Nevermania*, and *Gomphostilbia*. This research reported a new record of presence of *Wallacellum* in Bogor which was located on Java Island. The previous study done by Takaoka and Davies [14] reported that there were only three sub genera of *Simulium* in Java Island, namely sub genus *Simulium*, *Nevermania*, and *Gomphostilbia*. The sub genus *Walacellum* was originally found on Seram Island (Maluku) and Biak Island (Papua) as reported by Takaoka [15]. This sub genus was found on Java Island possibly because of its presence in Java Island that has not been explored and identified previously. The sub genus *Walacellum* was usually found in a wide river basin and no other species are found simultaneously with *Simulium* (*Wallacellum*) sp. from one river [15]. This was in accordance with river conditions where the sub genus *Walacellum* was found at this research site which has river width of 9 m, and is the only *Simulium* found in Leuwi Hejo Waterfall and Barong Waterfall.

The highest water temperature at all of the observed waterfalls was 23.17 ºC, while the lowest temperature was 18.19 ºC. The water temperature was suitable for the development of *Simulium* larvae, as stated by Hadi [18] that the optimum temperature for the development of *Simulium* larvae was 16-28 ºC, whereas according to Crosskey [3] the black fly was found in waters with 0-35 ºC. Based on the analysis results, the water temperature has a significant relationship to the number of *Simulium* larvae found with negative correlation (r = -0.423 and p = 0.036). The negative correlation indicates that the higher the water temperature the smaller number of *Simulium* larvae was found. Several previous studies have also reported negative correlation results between temperature and number of *Simulium* found, as reported by several [19-21]. Water temperature was influenced by several things such as air temperature, soil temperature and hydrological impairment produced naturally or by human intervention [22]. Meanwhile, Srisuka *et al*. [23] reported that black flies found more in the winter season compared to summer because during winter temperatures also decrease so that the water temperature around the river also decreases. In the contrary, other researchers reported different results that in Northern Thailand and Southern Thailand the black flies found in summer [24-25]. The pH value used as a measurement of the degree of acidity or alkalinity and the concentration of hydrogen ions in the water. The pH value of water in 19 waterfalls around Bogor Forest Management Unit has a diverse value. The highest pH was 8.14, while the lowest pH was 4.61. Neutral pH was an area favoured by *Simulium*, but some species can live in water with a pH above and below neutral, ranging from 6.56-8.90 [26]. Changes in pH value can affect aquatic life indirectly by changing the chemical aspect [27]. In this study, there was no significant relationship between water pH and the number of adult *Simulium* larvae found. This was consistent with previous studies suggesting that pH was an important factor for larval ecology that was difficult to assess, and a factor that was not particularly significant in sibling species [3]. It was stated by Opoku [28] that the pH value of the river is affected by stones, soil, and rotting plants around the stream. Rotting plants reduce dissolved oxygen levels, thus affecting the type and amount of black flies found.

The depth of the water referred in this study was the depth of water where larvae of *Simulium* discovered and pupae of *Simulium* attached to water plants, stones, stems or leaves. The depth of water in this study ranged from 6.68-93.3 cm. The water depth range is suitable for the proliferation of *Simulium* larvae and this result was supported by previous studies of Iboh and Arong [29] that found larvae and pupae of black flies at 6 cm depth. Coscaron and Coscaron Arias [26] found larvae and pupae of *Simulium* at depth no more than 50 cm, while Hamid *et al*. [30] found larvae and pupae of Simuliidae at a depth of less than 100 cm. The stream depth in this study also did not show significant results with the number of *Simulium* larvae found.

The stream width in this study ranges from 0.5-9 m. Black fly larvae can be found at a very wide diversity of streamlines, such as Yaqob *et al*. [21] had found *Simulium* larvae in rivers

with a flow width of 0.05-15 m, while Srisuka *et al*. [23] found *Simulium* larvae with a flow width of 0.47-1.08 m. The number of *Simulium* larvae and stream width have a significant relationship and have negative correlation value (r = -0.411 and p = 0.004). A negative correlation means that the wider the stream of the river the smaller the number of *Simulium* larvae found. The wider the stream of the river, the flow rate will slow down, so that the less the number of *Simulium* larvae found. The results of this study contradicted previous research conducted by Tongjura *et al*. [20] which showed a negative and positive correlation between the number of *Simulium* larvae found with flow width, where black fly larvae can grow both in wide and narrow rivers.

The highest velocity at the study site was 96.97 cm/sec. Meanwhile, the slowest velocity was 31.51 cm/sec. The minimum velocity that can be used by black fly to survive was 50 cm/sec [31]. Black fly larvae in some areas can also be found in streams at a speed of 5-350 cm/sec [3]. Significant value and positive correlation were obtained from the relation of the number of *Simulium* larvae with velocity (r = 0.557 and p = 0.007). Positive correlation value has a one-way meaning that the faster the flow of water the more the number of larvae *Simulium* found. These results were supported by previous research [32, 23, 21]. Gersabeck and Merriet [33] suggested that flow velocity was an important factor in the micro distribution of most larvae in river flows. The faster the flow of the river, the more the availability of food and oxygen to survive so the more number *Simulium* larvae found. This is supported by previous research by Onojafe *et al*. [34]

Elevation of 19 waterfalls which became the location of this research ranged between 483-1368 metres above sea level. Meanwhile, based on the results of correlation analysis, no significant relationship was found between the altitude with the number of Simulium larvae found. Non-significant associations were also reported in previous studies [19, 35]. This was inconsistent with other studies suggesting that altitude was correlated with the amount of *Simulium* found [23, 25]. Meanwhile, Hadi [18] reported the population of the *Simulium* fly differently every month and at different altitudes.

The canopy cover in this study was determined by the number of trees around the waterfall. Significant value and positive correlation were obtained from the relation of the number of *Simulium* larvae to the canopy cover (r = 0.548 and p = 0.008). Positive correlation value has a unidirectional meaning that the more canopy cover around the waterfall the more number of *Simulium* larvae found. The result was in agreement with the results of previous study by Ya'cob *et al*. [21]. Meanwhile, Srisuka *et al*. [23] found different results, ie the presence of shading with the amount of *Simulium* obtained has a negative correlation. Almet [36] states that the more canopy cover area around the breeding sites of *Simulium* the higher the number of *Simulium* found. It happened because more leaves were available as breeding sites. The more number of shading can also affect the water temperature. The presence of more shading causes the area of the river exposed to less sunlight so the colder the environmental temperature. The results of this study were in line with previous research that the presence of a slight shading resulted in an increase in water temperature, resulting in the lower number of black flies found [37].

Result of correlation test between the number of plants on the edge of the flow with the number of *Simulium* larvae found was not significant. This probably occurred because of the difference in the time of sampling. Research sampling was done during rainy and dry season. Sampling conducted during low rainfall resulted in reduced water flow so that the riparian vegetation of the river flow were not affected by the river flow. Low rainfall also causes reduced flow velocity due to reduced water flow [39]. Previous studies have reported that the more number of *Simulium* obtained in the rainy season than in the dry season [39, 19, 23]. Contrarily, Eugene [40] reported that a huge number of *Simulium* found in the dry season.

5. Conclusion

From the present study it can be concluded that the river flows around the waterfall areas of Bogor Forest Management Unit served as a suitable breeding habitat of the immature stage of *Simulium*. The result data of this research can be used for the research of other environmental factors that affect the population and distribution of *Simulium* in Indonesia.

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