Development of *Aedes spp* Larvae in Media with Various pH and Salinity: Study on Experiment Approach in Laboratory

Dwi Umeisyaroh[1], *Martini* Martini[1], *Ari* Udijono[1], Retno Hestiningsih[1*], *Henry* Setiawan Susanto[1], and *Sri* Yuliawati[1]

[1]Department of Epidemiology and Tropical Desease Faculty of Public Health, Diponegoro University, Indonesia

Abstract. The development of *Aedes spp*, a type of mosquito, is closely tied to the water. They can adapt to different aquatic environments regardless of their characteristics. Understanding the traits of *Aedes spp* and the environmental factors that affect them is crucial for vector control. This study aimed to examine the impact of water acidity and salinity on the development of pre-imago *Aedes spp*. The observed developments included hatchability of eggs, ability to change from larvae to pupa (ecdysis), transformation from pupa to imago (explosion), and the longevity of the pre-imago stage. The research followed an experimental approach with a post-test only control group. Mosquito eggs obtained from Semarang Port Health Office Laboratory Semarang were used as research subjects. Data analysis was conducted using the Kruskal-Wallis test followed by the Mann-Whitney test. The tests for pre-imago development were carried out at different average acidity levels (5.2, 6.6, 7.2, 7.4, 8.5, and 9.3) and average salinity levels (0‰, 2‰, 4‰, 6‰, 8‰, 10‰), with four replications. The analysis revealed that there were no significant differences in the development of pre-imago *Aedes spp* under various water acidity conditions (egg hatchability p-value = 0.736, ecdysis p-value = 0.814, explosion p-value = 0.591, longevity p-value = 0.227). However, the salinity analysis indicated different developments in pre-imago *Aedes spp* under varying salinity conditions (egg hatchability p-value = 0.006, ecdysis p-value = 0.001, explosion p-value = 0.001, longevity p-value = 0.003).

1 Introduction

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus. The *Aedes spp.* mosquito is known as the vector for transmission of the dengue virus and is regarded as the fastest-growing mosquito in the world, responsible for nearly 390 million infections annually. DHF poses a significant public health challenge as the number of cases continues to rise and its geographical spread widens [1].

* Corresponding author: retnohestiningsih@gmail.com

In 2019, DHF cases were reported in 34 provinces across Indonesia, with the highest occurrences in West Java (23,483 cases), East Java (18,031 cases), and Central Java (9,124 cases). Notably, the highest number of DHF-related fatalities were recorded in East Java (285 deaths) and Central Java (123 deaths) [2]. The morbidity rate for DHF in Central Java in 2019 was 26.15 per 100,000 people, reflecting a substantial increase from the 2018 rate of 10.20 per 100,000 people. Furthermore, the mortality rate for DHF in 2019 stood at 1.41%, representing an increase from 1.24% in 2017 and 1.05% in 2018.

It is important to note that all regencies/cities in Central Java Province are endemic areas for DHF, raising concerns about the potential for an increase in cases at any given time [3, 4]. The transmission of dengue fever is a result of the interplay between mosquitoes, dengue viruses, and human hosts, all of which are influenced by various environmental factors such as biological, physical, chemical, and host immunity [1]. Research has revealed that *Aedes spp.* can adapt to unconventional conditions, as *Aedes aegypti* is capable of breeding in coastal areas with brackish water sources and a pH range of 3-12.2 Despite efforts, control measures have not effectively prevented dengue fever cases, as the larvae-free rate, a crucial indicator, has not met the target of ≥ 95% since 2010 [3, 4].

Control strategies have traditionally targeted freshwater containers around settlements, overlooking *Aedes spp.*'s adaptation to environments such as gutters and tidal water puddles. Heightened vigilance regarding stagnant water, including wastewater channels, tidal pools, and former ponds, is imperative for eradicating mosquito breeding sites [5–7]. Moreover, research has been conducted to explore the impact of varying pH and water salinity conditions on the development of pre-adult *Aedes spp.*

2 Method

The study employed an experimental approach utilizing a posttest-only control group design. The study focused on the independent variables of water pH, encompassing concentrations of 5.2, 6.6, 7.2, 7.4, 8.5, and 9.3, alongside water salinity with concentrations of 0‰, 2‰, 4‰, 6‰, 8‰, and 10‰. Control conditions were established using distilled water with a pH of 7.2 and salinity of 0‰. The study examined the development of pre-adult *Aedes spp.*, including hatching power, ecdysis ability (transition from larvae to pupae), exclosion ability (transition from pupae to imago), and the development time of eggs to imago. This research was undertaken at the Semarang Port Health Office Laboratory, and replication was determined utilizing the Federer formula (t-1)(r-1) ≥ 15, resulting in four replications. Study subjects consisted of eggs obtained from mosquitoes bred at the Semarang Port Health Office Laboratory. Data analysis was conducted using the Kruskal-Wallis test, followed by the Mann-Whitney test.

The study started with setting up ovitraps to collect mosquito eggs. The eggs were then maintained in a 30x30x30 cm cage in the laboratory. The collected eggs were put into different pH and salinity solutions to observe their development. Acidic pH solutions were made using 0.1 M HCl, while alkaline pH solutions were made using 0.1 M NaOH.

Salinity solutions were made using NaCl. A total of 48 ovitraps were prepared with these solutions.

Measurements of pH concentration, salinity, and other environmental factors were recorded every 6 hours over a 28-day observation period. The hatching rate was calculated by comparing the number of hatched eggs to the initial number of eggs. Eggs that didn't hatch within 17 days were considered non-hatched. The percentage of larvae successfully becoming pupae was calculated for each treatment. Similarly, the percentage of pupae becoming adult mosquitoes (imago) was also calculated. Finally, the time taken for the eggs to develop into adult mosquitoes was recorded.

3 Results

3.1 Development of Pre-Adult *Aedes spp* in Various Water pH Conditions

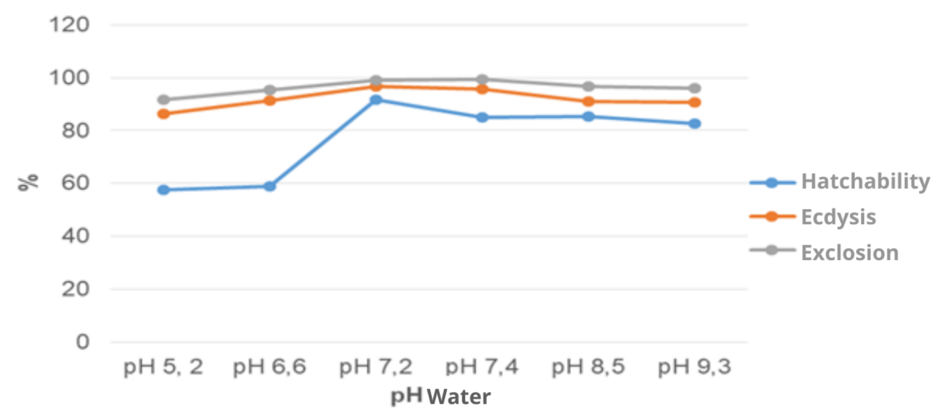

Figure 1. Percentage of Hatchability, Ecdysis Ability, Exclosion Ability of *Aedes spp* in Various Water pH Conditions

In Figure 1, it is evident that the egg hatching power, ecdysis ability, and exclosion ability of *Aedes spp*. decrease as the pH becomes more acidic or alkaline. Over 90% of pupae in all treatments successfully developed into adults. The optimal development of pre-adult *Aedes spp*. occurred at pH 7.2. The percentage of ecdysis and exclosion ability was lowest at pH 5.2, while it remained high at pH 9.3. The Kruskal-Wallis test results indicated no significant difference in water pH concentration on hatching power (p-value = 0.736), ecdysis ability (p-value = 0.814), and exclosion ability (p-value = 0.591). Additionally, the development time was observed during the hatching power, ecdysis, and exclosion processes with specific results.

Table 1. Development of Pre-Adult *Aedes spp* in Various Water pH Conditions.

pH Concentration	Pre-adult Development (Days)			Average Progress (Days)
	Egg-Larva	Larva-Pupa	Pupa-Imago	
5,2	8	6,2	1,7	11,7
6,6	2,7	5,4	2	10,1
7,2	2	6	1,3	9,3
7,4	1,6	6	1,6	9,2
8,5	2,3	5,8	1,6	9,7
9,3	1,8	6,2	1,7	9,7

In Table 1, the development of the pre-adult stage was longest at pH 5.2 and fastest at pH 7.4. The larvae developed into imago within 7.3 - 7.9 days. The Kruskal-Wallis test results indicated no significant difference in the development time of pre-adult *Aedes spp* in various water pH conditions, with a p-value of 0.227. The statistical analysis showed no difference in hatching power, ecdysiast ability, exclusions ability, and development time. Therefore, it can be inferred that there is no significant difference in the development of pre-adult *Aedes spp* in various water pH conditions.

3.2 Development of Pre-Adult *Aedes spp* in Various Water pH Conditions

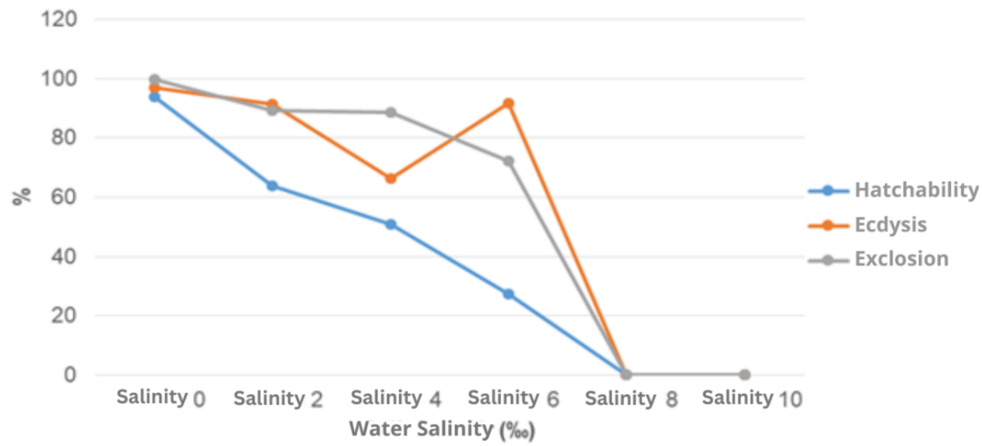

Figure 2. Percentage of Hatchability, Ecdysis Ability, Exclosion of *Aedes spp* in Various Water Salinity Conditions

Figure 2 illustrates that as the salinity concentration increases, the ability of the eggs to hatch, undergo ecdysis, and complete exclosion decreases. Over the course of 18 days, no larvae hatched from eggs in 8‰ and 10‰ salinity. The percentage of ecdysis ability at 6‰ salinity was higher compared to 2‰ and 4‰ salinity. Statistical analysis indicated a significant difference in water salinity concentration on hatching power (p-value = 0.006), ecdysis ability (p-value = 0.001), and exclosion ability (p-value = 0.001). In addition, observations were made regarding development time, yielding the following results:

Table 2. Development of Pre-Adult *Aedes spp.* in Various Water Salinity Conditions.

Salinity Concentration (‰)	Pre-adult Development (Days)			Average Progress (Days)
	Egg-Larva	Larva-Pupa	Pupa-Imago	
0	2,2	5,6	1,5	9,3
2	3,4	5,7	1,6	10,7
4	4,1	5,6	1,8	11,5
6	5,3	6,2	2,5	11,8
8	0	0	0	0
10	0	0	0	0

Table 2 indicates that higher salinity concentrations lead to longer pre-adult stage development. *Aedes spp* showed the longest development time at a salinity of 6‰, averaging 11.8 days. Statistical analysis revealed differences in pre-adult *Aedes spp* development times under various water salinity conditions, with a p-value of 0.003. The analysis also showed significant differences in hatching power, ecdysis ability, exclosion ability, and development time, indicating variations in pre-adult *Aedes spp* development under different water pH conditions. The Mann-Whitney test was used to identify differences at each salinity concentration, revealing significant discrepancies in egg hatching power at salinity concentrations of 8‰ and 10‰, as well as in the development time of the pre-adult stage at 4‰ salinity.

4 Discussion

The results of the study indicated that *Aedes spp* could thrive in water with a pH of up to 9.3. Aggraini's 2017 study demonstrated that *Aedes aegypti* can develop well at pH 9, with the highest occurrence of instar II larvae development observed at this pH level [8]. However, another study contradicted this, stating that larvae cannot develop at pH 9.3 due to high surfactant content, which interferes with oxygen uptake and inhibits cell growth. In the latter study, distilled water with added NaOH was used as the development medium to control other factors that could affect larval respiration.

The longest pre-adult stage development was seen at pH 5.2, while the fastest development occurred at pH 7.4. The process of larval development triggers the release of juvenile and ecdysone hormones, and a disrupted skin change process due to water pH affecting these hormones may lead to prolonged development time and higher larval mortality rates [9]. Latorre et al. noted that the mortality rate of *Aedes aegypti* larvae is relatively higher in an acidic environment (pH 4) compared to a neutral or slightly alkaline environment. This suggests that acidic pH levels are not optimal for the survival of *Aedes aegypti* [10].

The optimal fecundity of *Aedes spp* eggs was between 2-7 days, but they could still hatch well up to 2.5 months. In this study, the eggs were hatched on the 5th day. Before hatching, the eggs were stored in a box equipped with silica gel, protected from predators, and kept at room temperature to meet the criteria for optimal fecundity. Media with an acidic pH could affect the hatching of eggs into larvae. Suparyati's 2021 study proved that the more acidic or lower the pH, the lower the hatching power of mosquito eggs [11]. Statistical analysis showed no difference in egg-hatching power at various water pH levels. These results aligned with Nungki's 2018 study, which stated that there is no significant relationship between water pH and the presence of larvae [12]. Eggs that have hatched will go through four stages of development where changes in each instar are marked by skin peeling. The development of instars I to IV lasts around 5-6 days. The acidity of water greatly determines the growth or survival of *Aedes spp* larvae.

The ability of insects to molt and emerge from their pupal stage was lowest at pH 5.2 (acidic), and highest at pH 9.3 (alkaline). The emergence ability at all pH levels showed an increase, exceeding 90%. This is because the pupal stage has stronger resistance than the larval stage. Larvae obtain ions from the environment through food and absorb active ions through the anal papilla, while pupae are dormant phases that do not eat and are not actively moving, which reduces ion absorption [13].

The statistical analysis of the study showed that there was no difference in the development of *Aedes spp.* in various water pH conditions, which aligns with Susanti's 2017 study stating that pH levels are statistically unrelated to the presence of larvae. The decrease in water pH (acidity) increases dissolved oxygen levels and speeds up microbial development. Consequently, the need for oxygen increases while the actual levels of dissolved oxygen decrease. The lack of dissolved oxygen reduces the formation of cytochrome enzymes involved in the metabolic process, resulting in lower ability for insects to molt and emerge as the pH decreases [14].

Statistical analysis has revealed significant differences in the hatching power of *Aedes spp* eggs under various water salinity conditions, with a p-value of 0.006. It is evident that the chlorine (Cl) content in water can disrupt the development and hatching process of mosquito eggs.8 Moreover, the ecdysis ability at a salinity of 6‰ surpasses that at salinities of 2‰ and 4‰, with statistical analysis highlighting differences in the ecdysis ability of *Aedes spp* larvae under various water salinity conditions, with a p-value of 0.001. This observation aligns with the research by Anggraini, which suggests that *Aedes aegypti* can progress to adulthood at a salinity of 4 - 6 ‰. Additionally, at a 6‰ salinity level, mosquito larvae exhibit notably larger growth.8 The adaptability of Aedes genus larvae to brackish conditions and varying salinities is evident through their regulation of ion levels via the excretory system to modulate the osmotic pressure of the hemolymph and adjust to the surrounding osmotic pressure [15].

Statistical analysis indicated that there were differences in the ability of *Aedes spp* pupae to survive in different water salinity conditions. This finding was consistent with the one of Marylene et al., suggesting that the higher the salinity level, the more significant the decrease in the survival of *Aedes spp.* [16]. During the pupal phase, which is a dormant phase characterized by no eating and minimal movement, osmotic pressure affects the physiological ability of the pupa. Higher osmotic pressure leads to increased energy expenditure to maintain physiological ability, which in turn affects the pupa's ability to survive. Salinity exposure begins at the egg phase, and the eggs that hatch into larvae must adapt to water pressure for approximately 7 days. Ultimately, there is a limit to this adaptation, and at higher pressures, the mortality rate of pupae is higher.

In this study, the pre-adult stage development period ranged from 9.3 to 11.8 days. The higher the salinity concentration, the longer it took for the pre-adult process to complete. These findings align with Rao's research in India, which indicated a positive correlation between salinity levels and density. *Aedes aegypti* was observed to develop into an adult mosquito at a water salinity concentration of 4 to 6 ‰. However, at a concentration of 6 ‰, the larvae were able to survive for up to 23 days without metamorphosing into pupae [17]. In higher salinity conditions, larvae may struggle to assimilate nutrients and expend more energy to maintain physiological processes, leading to a slower development time [16].

The *Aedes aegypti* mosquitoes are able to adapt to varying levels of salt in different environments such as coastal areas, beaches, and highlands. In areas with seawater intrusion, the water used for daily activities has been mixed with salt water [12]. Martini et al's research in North Semarang reveal significant changes in ion transport by the anal papillae of mosquito larvae living in salt water. *Aedes aegypti* mosquitoes have adapted to environments with high salt content (2-3‰) [2]. Research in Sri Lanka showed that high larval density in brackish coastal areas on the Jaffna Peninsula is positively correlated with increased incidences of dengue fever in parts of Jaffna City bordering the sea. Larvae can develop into adults and transmit the Dengue virus vertically in brackish water up to 10‰ [18].

In this study, it was observed that pre-adult *Aedes spp* showed different rates of development in salinity conditions ranging from 0‰ to 6‰. At a salinity of 6‰, the eggs were able to hatch and develop into adults. The highest molting ability was observed at a salinity of 6‰. It is essential to evaluate coastal areas where seawater intrusion has affected wells, PDAM networks, and areas previously believed to be free of *Aedes spp*, such as stagnant sewer water, old ponds, and water containers impacted by tidal inundation. Control efforts have primarily focused on freshwater areas near human habitation, but rising sea levels due to global warming may expand natural brackish water bodies in coastal areas, leading to an increase in salinity-tolerant mosquito vectors and elevated disease transmission [7].

The development of Aedes mosquitoes in nature is influenced by various factors including pH, salinity, temperature, humidity, compounds in water such as CO2, dissolved oxygen levels, ammonia, salt concentration, organic matter decomposition at the bottom of the water, rainfall, seasonal changes, and the presence of microorganisms [19]. These factors directly affect pH stability. The concentration of pH and salinity are important indicators that affect the survival of organisms in water [20]. Research by Martini et al in 2017 demonstrated that Aedes mosquitoes can develop in wastewater with a pH of 8.7. Additionally, research by Jacob et al in 2014 showed that Aedes mosquitoes can develop in clear sewer water. Furthermore, a study conducted on the coast of Jaffna City found eggs and larvae of *Aedes aegypti* and *Aedes albopictus* in discarded plastic and glass containers, abandoned fishing boats, and unused wells, where the water type in these places is brackish water [7].

This study found that there is no significant difference in the development of pre-adult *Aedes spp* in varying water pH conditions. These results suggest that both acidic and alkaline pH levels have the potential to increase the risk of *Aedes spp* developing and becoming a vector. This finding is in line with Yuniar's research, which indicates that household waste and PDAM water are the most preferred and optimal places for the development of *Aedes aegypti* larvae into adult mosquitoes [9]. Therefore, controlling *Aedes spp*, especially in the pre-adult stage, cannot be accomplished solely by focusing on clean water containers, both inside and outside the house.

When measuring the water at the location of the ovitrap installation, all containers observed were alkaline. When the pH concentration of PDAM water was examined, it was found that the most alkaline measurement was at pH 8. In alkaline water conditions, the concentration of OH- ions was greater than that of OH +, and the levels of dissolved oxygen were lower. As a result, microbial growth was relatively normal and the chance of survival was greater. A pH of 7.3 - 8 was identified as the optimal condition for larval development.

The results of this study confirmed that the highest hatching, ecdysis, and exclosion occurred in the pH range of 7.1 - 8. At this pH range, the chance of survival during the pre-adult stage was higher, and the life cycle was more stable. The water in the container at the ovitrap installation location was measured and found to be brackish due to a salinity concentration of ≥ 0.5. The study results indicated that the ability of *Aedes spp.* to molt and emerge at a salinity of 1.1 - 3 was the highest, with a percentage of over 89 - 92%. The measurement results also showed that the salinity concentration could support the development of *Aedes spp.* At a salinity of 6‰, *Aedes spp.* eggs could still hatch and developed into adult mosquitoes. The water observed appears clear, calm (not flowing), and was protected from sunlight due to semi-closed gutters and the presence of many trees.

The results of this laboratory study demonstrated the adaptability of *Aedes spp* to non-optimal pH and salinity. However, in their natural environment, the development of *Aedes spp* was significantly influenced by various physical, biological, and chemical factors. It was important to note that development at the same pH in the laboratory and in nature might differ. Nevertheless, the finding that the concentration of pH and salinity could support the development of *Aedes spp.* should be considered when implementing effective control measures to reduce the transmission of dengue fever.

5 Conclusion

Aedes spp. exhibit reduced development at higher water salinity or at non-normal pH levels. However, their ability to breed at pH 5 and salinity 6‰ demonstrates their resilience even under suboptimal conditions. These results underscore the crucial need for vigilance and an early warning system among program managers and the community to control areas previously believed to be free of *Aedes spp.*, such as stagnant water channels, water containers from tidal flooding, and former ponds. Future research should investigate the relationship between pH and salinity in the life cycle of *Aedes spp.*

References

1. Sub Direktorat Vektor dan Binatang Pembawa Penyakit, Pedoman Survei Entomologi Demam Berdarah Dengue dan Kunci Identifikasi Nyamuk Aedes (2017).
2. M. Martini, Incidence of Dengue Hemorrhagic Fever (Dhf) in Semarang Coastal Area: Epidemiology Descriptive Case and Bionomic Vector. Indones. J. Trop. Infect. Dis. **7**, 144 (2019).
3. Kementerian Kesehatan RI, InfoDatin Situasi Demam Berdarah Dengue di Indonesia Tahun 2018 (2018).
4. Kementerian Kesehatan RI, Permenkes No. 50 Tahun 2017 tentang Standar Baku Mutu Kesehatan Lingkungan dan Persyaratan Kesehatan Untuk Vektor dan Binatang Pembawa Penyakit Serta Pengendaliannya (2020).
5. H. I. Adifian & R. La Ane, Kemampuan Adaptasi Nyamuk Aedes Aegypti dan Aedes Albopictus dalam Berkembang Biak Berdasarkan Jenis Air. Appl. Surf. Sci. 467–468 (2019).
6. J. A., V. Pijoh, & G. Wahongan, Ketahanan Hidup dan Pertumbuhan Nyamuk Aedes sp pada Berbagai Jenis Air Perindukan. J. e-Biomedik **2** (2014).
7. R. Ramasamy, S. N. Surendran, P. J. Jude, S. Dharshini, & M. Vinobaba, Larval development of Aedes aegypti and Aedes albopictus in peri-urban brackish water and its implications for transmission of arboviral diseases. PLoS Negl. Trop. Dis. **5** (2011).
8. T. S. Anggraini & W. H. Cahyati, Perkembangan Aedes Aegypti Pada Berbagai pH Air dan Salinitas Air. Higeia **1**, 1–10 (2017).
9. M. Martini, Y. Triasputri, R. Hestiningsih, S. Yuliawati, & S. Purwantisasi, Longevity and development of Aedes aegypti larvae to imago in domestic sewage water. J. thee Med. Sci. (Berkala Ilmu Kedokteran) **51**, 325–332 (2019).
10. M. Latore, B. Morejon, & M. Neira, The Impact of Rearing Temperature and Water pH on Longevity and Pesticide Resistance in Aedes Aegypti (Diptera: Culicidae). Trends Genet. **35**, 470–1 (2018).
11. T. Suparyati & M. D. Himam, Daya Tetas Telur Nyamuk Aedes Aegypti pada Tiga Jenis Air Perindukan Di Kelurahan Medono Kota Pekalongan. Pena J. Ilmu Pengetah. dan Teknol. **35**, 61 (2021).
12. N. H. Suryaningtyas, I. Margarethy, & D. Asyati, Karakteristik Habitat Dan Kualitas Air Terhadap Keberadaan Jentik Aedes Spp Di Kelurahan Sukarami Palembang. Spirakel **9**, 53–59 (2017).
13. M. A. Albers & T. Bradley, On the Evolution of Saline Tolerance in the Larvae of Mosquitoes in the Genus Ochlerotatus. Physiol. Biochem. Zool. **84**, 258–267 (2011).
14. S. Susanti & S. Suharyo, Hubungan Lingkungan Fisik Dengan Keberadaan Jentik Aedes Pada Area Bervegetasi Pohon Pisang. Unnes J. Public Heal. **6**, 271–276 (2017).
15. J. D. Galavíz-Parada, F. Vega-villasante, S. Guerrero-galván, & J. L. N. Heredia, Efecto de la temperatura y salinidad en la eclosión y supervivencia de Aedes aegypti. Rev. Cubana Med. Trop. **71**, 1–14 (2019).
16. M. De Brito Arduino, L. F. Mucci, L. L. N. Serpa, & M. De Moura Rodrigues, Effect of salinity on the behavior of Aedes aegypti populations from the coast and plateau of southeastern Brazil. J. Vector Borne Dis. **52**, 79–87 (2015).
17. B. Bhaskar Rao, P. S. Harikumar, T. Jayakrishnan, & B. George, Characteristics of Aedes (Stegomyia) albopictus Skuse (Diptera: Culicidae) breeding sites. Southeast Asian J. Trop. Med. Public Health **42**, 1077–1082 (2011).
18. P. J. Jude et al., Salinity-tolerant larvae of mosquito vectors in the tropical coast of Jaffna, Sri Lanka and the effect of salinity on the toxicity of Bacillus thuringiensis to Aedes

aegypti larvae. Parasites and Vectors **5**, 2–9 (2012).
19. M. Sallata, E. Ibrahim, & M. Selomo, Hubungan Karakteristik Lingkungan Fisik dan Kimia dengan Keberadaan Larva Aedes aegypti di Wilayah Endemis DBD Kota Makassar. Univ Hasanuddin 1–10 (2013).
20. K. Amri, M. Muchlizar, & A. Ma'mun, Variasi Bulanan Salinitas, Ph, Dan Oksigen Terlarut Di Perairan Estuari Bengkalis. Maj. Ilm. Globe **20**, 58 (2018).