Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.512

Research Article

Hatching Ratio and Larval Development of *Aedes aegypti* Eggs in Different Growth Media

M. Ali Azis Hasan Rizki[1*] Dadan Supardan[2]

[1]*Universitas Islam Al Azhar, Mataram, Nusa Tenggara Barat, Indonesia*
[2]*Institut Agama Islam Negeri Curup, Bengkulu, Indonesia*

**Corresponding author: hasanrizki@unizar.ac.id*

Abstract

Aedes aegypti is a mosquito species primarily known as the vector for dengue fever. The Hatching and development larval of *Aedes agypti* are crtical factors in mosquito control strategies and reducing dengue fever transmission. This study aims to investigate the effects of different water media on the hatching rate and development of *Aedes aegypti* from egg to adult stage. Three type of water from well water, treated tap water (PDAM) and staw soaked water were used in the experiment. The selection of water sources was based on their distinct physical and chemical properties, representing common environmental condition where *Aedes aegypti* may breed. Straw soak water was incorporated to simulate organic rich aquatic environments, often characterized by the presence of decomposing plant material. Such conditions are known provide a nutrient rich medium that facilitates mosquito egg hatching and larval development. Eggs were placed in each water type, and observations were carried out at hatching rate and larval development. The result revealed that the straw soaked water had the highest hatching rate 100% and the development time 6 days from egg to adult, well water show a hatching rate 67% with a longer development period of 7 days and no hatching and larval developmet in tap water (PDAM). This study shows that various types of water media affect the hatching success and developmental rates of *Aedes aegypti.* However, these effects are limited to the specific water types used in the experiment and should not be assumed to apply to all water quality conditions. Future research is recommended to explore other water quality parameters and their potential impact on the mosquito life cycle.

Keywords: *Aedes aegypti*, Hatching rate, larval development, Straw-soaked water, Water quality

1. Introduction

Vector-borne disease transmission dengue fever is a major concern for public health. Every year, 96 million cases of dengue fever occur worldwide causing approximately 9.110 deaths (Budiman et al. 2021). Indonesia has faced significant challenges with dengue fever, with case numbers fluctuating highly over recent years. In 2024, Indonesian ministry of health reported 88,593 cases and 621 deaths (WHO, 2024). Effective vector control strategies and a comprehensive understanding of the mosquito life cycle from egg to adult are essential to reduce dengue fever transmission. Knowledge of the mosquito life cycle is essential for developing effective prevention methods (Buhler et al. 2019).

The life cycle of *Aedes aegypti* includes several stages from egg , four larval instars, pupa and adult. Each stage is influenced by environmental factors such as water quality, temperature, humidity, survival, and population density (Arévalo-Cortés et al. 2022). The effective incubation of eggs and larval development is highly influenced by the type of water used for development, organic matter, and pH. Eggs of *Aedes aegypti* can remain

viable in dry conditions and hatch upon submersion in water, the quality of water significantly affects hatching success and development timing (Soares-Pinheiro et al. 2017). After hatching, the larvae pass through four stages to reach the instar before becoming to pupae. Larval ontogeny develops most rapidly and uniformly in media a with high organic matter (Tsegaye et al. 2023).

Recent study highlight the important role of water quality and type of water development *Aedes aegypti*. The type of water, such as rain water and treated tap water, which have different organic matter composition, has been shown to affect hatching success and larval survival (Thia Prameswarie et al. 2023). Furthermore, envronmental factors such as temperature and nutrient availability are crucial in determining how effectively mosquitoes develop at each stage(Souza et al. 2019).

This paper aims to explore how different types of water media affect the hatching rate and larval development of *Aedes aegypti*. This study investigates these dynamics to provide insights that can improve mosquito control strategies and reduce the risk of vector-borne disease transmission. The concept that various types of water affect larval development can lead to more effective control, ultimately reducing dengue incidence and enhancing public health outcomes.

2. Material and Method

Sample Collection

Aedes aegypti eggs were obtained and maintained in the Biology Education laboratory, UIN Mataram. The water types were kept under controlled conditions with a temperature of 29 ± 1°C and a Ph of ± 8.0.

Experimental Design

Three types of water were used in the development of *Aedes aegypti:* well water, tap water (PDAM) and Straw-soak water. Each type of water was tested in triplicate to ensure reproducibility. A total of 1 L experimental units was prepared, with each unit consisting of a container filled with 50mL of the respective water type.

Procedure

A standardized 10 number of eggs was placed into each container of water, the container were convered with mesh to prevent contamination and loss of eggs. The containers were observation, parameters recorded included the following. The number of eggs that hatched within a specified time frame. Then, the progression of larvae through the instars (1^{st} to 4^{th}) was observation. Measurement included the duration of each instar and survival rate. The transition from larva to pupa and to adult mosquito was recorded.

Statistical Analysis

The data collected from the experiments were analyzed using SPSS. Statistical tests, ANOVA and Tukey's HSD, were performed to compare hatching rates, development times,

and survival rates across different water media. A significance level of $p < 0.05$ was used for all statistical analyses

3. Results and Discussion

3.1. Results

Table 1. Hatching Ratio and Developmental stage *Aedes aegyptiI* (Analysis of Variance One Way)

Variable	Sum of Squares	df	Mean Square	F-Value	P-Value
Hatching Ratio	13333.34	2	6666.67	20.0	0.004
Duration Egg to Instar 1	6.802	2	3.401	5.3	0.05
Duration Instar 1 to Pupa	33.33	2	16.667	125	0
Duration Pupa to Adult	1.052	2	0.526	15.781	0.007

The ANOVA results reveal significant differences in several developmental variables of *Aedes aegypti* based on the type of water used. For the Hatching Ratio variable, an F Value of 20.0 with a p-value of 0.004 indicates that the type of water significantly affects the egg hatching ratio. This suggests that different water types impact the hatching rate of the eggs. For the Duration Egg to Instar 1 variable, the F Value of 5.3 and a p-value of 0.05 suggest marginal differences in the duration from egg to Instar 1 among the tested water types. Although this difference is close to the threshold of significance, further evaluation is warranted. The Duration Instar 1 to Pupa variable shows a very high F Value of 125.00 with a p-value of 0, indicating a highly significant difference in the duration of development from Instar 1 to pupa. This suggests that water type has a substantial impact on the rate of larval development during this phase. Finally, for the Duration Pupa to Adult variable, the F Value of 15.781 and p-value of 0.007 indicate significant differences in the duration from pupa to adult mosquito between water types. Overall, these results confirm that the type of water significantly influences both the hatching ratio and the duration of various developmental stages of *Aedes aegypti*.

Table 2. Hathing Ration and Developmental Stage (Posthoc Test Tukey-HSD)

Variable	**Comparison**	**Mean Difference**	**P-Value**
Hatching Ratio	Well Water - Staw Soak Water	33.33	0.69
	Well Water - PDAM Water	66.67	0.015
	Straw Soak Water - PDAM Water	100	0.06
Duration Egg to Instar 1	Well Water - Staw Soak Water	0.167	0.97
	Well Water - PDAM Water	1.84	0.08

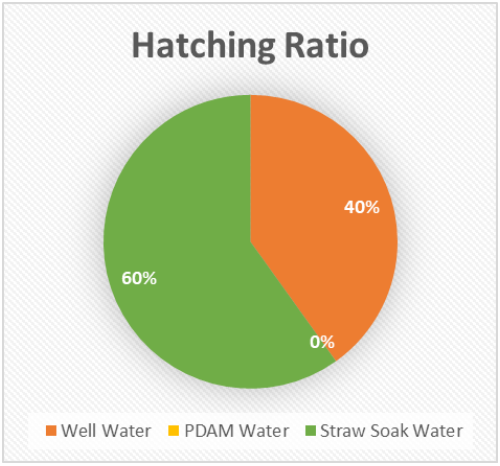
	Straw Soak Water - PDAM Water	2	0.08
Duration Instar 1 to Pupa	Well Water - Staw Soak Water	1.67	0.009
	Well Water - PDAM Water	4.67	0
	Straw Soak Water - PDAM Water	3	0.01
Duration Pupa to Adult	Well Water - Staw Soak Water	0.3	0.2
	Well Water - PDAM Water	0.8	0.006
	Straw Soak Water - PDAM Water	0.5	0.06

The post-hoc analysis for the hatching ratio reveals a significant difference between Well Water and PDAM Water, with a mean difference of 66.67 and a p-value of 0.015. This indicates that Well Water significantly supports a higher hatching ratio compared to PDAM Water. The comparison between Straw Soak Water and PDAM Water shows a mean difference of 100.00, though this difference is not statistically significant ($p = 0.06$). No significant difference was found between Well Water and Straw Soak Water (mean difference = 33.33, $p = 0.69$).

Regarding the duration from egg to Instar 1, no significant difference was observed between Well Water and Straw Soak Water (mean difference = 0.167, $p = 0.97$). However, there is a trend suggesting a longer duration in PDAM Water compared to Well Water (mean difference = 1.84, $p = 0.08$) and Straw Soak Water (mean difference = 2.00, $p = 0.08$), although these differences are not statistically significant. For the duration from Instar 1 to Pupa, significant differences are evident. Well Water shows a longer duration compared to Straw Soak Water, with a mean difference of 1.67 and a p-value of 0.009. Additionally, the duration in PDAM Water is significantly longer compared to both Well Water (mean difference = 4.67, $p < 0.001$) and Straw Soak Water (mean difference = 3.00, $p = 0.01$).

In terms of the duration from Pupa to Adult, Well Water exhibits a shorter duration compared to PDAM Water, with a mean difference of 0.8 and a p-value of 0.006, indicating a significant difference. The comparison between Straw Soak Water and PDAM Water approaches significance (mean difference = 0.5, $p = 0.06$), suggesting a trend towards a longer duration in PDAM Water. These results highlight significant differences in development rates and hatching ratios based on the type of water used, with Well Water generally supporting more favorable outcomes compared to PDAM Water.

Hatching Ratio



(**Figure 1.** Hatching Ratio)

The study reveals significant differences in the hatching ratios of *Aedes aegypti* eggs among various water media. In well water, the hatching ratio is 67%, indicating a relatively good rate of egg hatching and suggesting that well water supports egg hatching effectively. In contrast, no eggs hatched in PDAM water, resulting in a hatching ratio of 0%. This lack of hatching suggests that PDAM water does not support egg hatching, likely due to factors such as poor water quality or the presence of inhibiting substances. On the other hand, straw soak water demonstrated a hatching ratio of 100%, meaning all eggs placed in this medium successfully hatched. This indicates that straw soaking water is highly effective in supporting *Aedes aegypti* egg hatching, making it an exceptionally supportive medium for this purpose.

Larva Development Based on Medium Type

Table 3. Larvae Development Based on Medium Type

Medium Type	Stage	Average Duration (Day)
Well Water	Egg - Instar 1	2
	Instar 1- Instar 2	0.84
	Instar 2- Instar 3	0.84
	Instar 3 - Instar 4	1.34
	Instar 4 - Pupa	1.67
	Pupa - Adult	0.5
PDAM water	Egg - Instar 1	-
	Instar 1- Instar 2	-
	Instar 2- Instar 3	-
	Instar 3 - Instar 4	-
	Instar 4 - Pupa	-
	Pupa - Adult	-

Straw Soak Water	Egg - Instar 1	2
	Instar 1- Instar 2	1
	Instar 2- Instar 3	1
	Instar 3 - Instar 4	1
	Instar 4 - Pupa	0.5
	Pupa - Adult	0.5

In well water, the transition from egg to instar 1 takes 2 days, while the changes from instar 1 to instar 2 and from instar 2 to instar 3 each require 0.84 days. Moving from instar 3 to instar 4 takes slightly longer, approximately 1.34 days. The duration from instar 4 to the pupal stage is 1.67 days, and the pupal stage to adult mosquito is the shortest, at just 0.5 days. This indicates that the development of *Aedes aegypti* larvae in well water shows fairly consistent timing between instars, but requires a longer period for the transition from instar 4 to pupa compared to the transition from pupa to adult mosquito.

In contrast, PDAM water showed no data on larval development, suggesting that it is not suitable for *Aedes aegypti* larvae or contains factors that inhibit their growth. Concurrently, in straw soak water, the duration from egg to instar 1 is 2 days, consistent with well water. The transitions between instars (from instar 1 to instar 2, instar 2 to instar 3, and instar 3 to instar 4) all take 1 day each. The transition from instar 4 to pupa, as well as from pupa to adult mosquito, each takes 0.5 days. Thus, straw soak water provides more uniform development times between instars and quicker transitions from pupa to adult mosquito compared to well water. Overall, well water exhibits variable development durations with stable instar transitions but longer pupal periods, whereas straw soak water shows more consistent timing and faster transitions. PDAM water is unsuitable for larval development, suggesting it may hinder mosquito growth.

Comparasion of Development Time

Table 4. Comparasion of Development Time

Medium Type	Duration egg to Larva	Duration Larva to Pupa	Duration Pupa to Adult	Total Duration
Well Water	2	4.5	0.5	7
Straw Soak Water	2	3.5	0.5	6

The development of *Aedes aegypti* from egg to adult was evaluated in two types of water: well water and straw soak water. The duration from egg to larva was consistent across both media, with a time of 2 days. However, the duration from larva to pupa was significantly longer in well water, taking 4.5 days, compared to 3.5 days in straw soak water. This indicates that the transition from larva to pupa is slower in well water. Both media exhibited the same duration of 0.5 days for the transition from pupa to adult, showing no difference in this phase. Overall, the total development time from egg to adult

mosquito was shorter in straw soak water (6 days) compared to well water (7 days). This suggests that straw soak water supports faster development of *Aedes aegypti* compared to well water. Thus, while the initial and final stages of development are unaffected by the type of water, the intermediate stage from larva to pupa is more prolonged in well water, resulting in a longer overall development time.

3.2. Discussion

Study examines how different water media affect the development of *Aedes aegypti* from egg to adult. the results show of siginificant differences in hatching ratios and developmental times across various water types, which have implicaations for mosquito control and ecological research

Habitat Preferences for Hatching of *Aedes aegypti* Eggs

The results of this study indicate that *Aedes aegypti* eggs hatch effectively in well water, with a hatching ratio of 67%. This suggest that well water, although not fully sterile, possesses characteristics conducive to egg hatching. Suwartawan et al. (2022), in their study, it was established that *Aedes aegypti* generally prefers clean water for oviposition, and well water, which is relatively cleaner than domestic sewage, provides a more favorable environment for hatching. However, a hatching ratio that does not reach 100% may indicate the presence of inhibiting factors or less than optimal conditions in the well water that limit complete hatching.

The Inhibitory Effects in Treated Tap Water(PDAM)

The absence of hatching in tap water, hatching ratio: 0%, shows that the tap water does not support eggs hatching. Possibily, chemical substances such as chlorine act as inhibitors, preventing egg development even causing egg mortality. This experiment aligns with previous research, treated water containing inhibitory substance such as chlorine can significantly reduce hatching susccess (Fahri et al. 2019; Imam et al. 2014).

Organic-rich matter in straw soak water

With a hatching ratio of 100%, the characteristics of straw soak water demonstrate the highly supportive nature of this medium for eggs hatching. The presence of organic matter potentially creates an ideal environment for the development of *Aedes aegypti*. Nutriens released from straw supply additional resources that support egg development. The observation is consistent with research that suggests organic matter in water can enhance the attractiveness of such environments for oviposition and support higher hatching rates (Fahri et al. 2019; Pradani, Mutmainah, and Marlina 2023)

Effect of water characteristics on Hatching

The paper presents various significant findings in hatching ratios, depending on the characteristics of each water type. Organic-rich matter (Straw soak water) proved to be the most productive for egg hatching, while the tap water (PDAM) which contains

inhibitory substances, completely inhibited hatching. The result highlight effects of physical and chemical factors in water, such as oxygen, pH, organic matter, and inhibitory substances in determining *Aedes aegypti* egg hatching. (Imam et al. 2014; Suwartawan et al. 2022)

Larval development in well water

The results indicate that *Aedes aegypti* larvae in well water relatively stable development between instars, with a duration of 2 days from egg to instar 1. However, the development becomes variable in the next stage, especially during transition from instar 4 to pupae (1,67 days) and from pupa to adult, which only 0,5 days. The variation probably related to the availability of nutrients in well water (Araújo, Gil, and E-Silva 2012). The amount of available nutrients significantly influences the rate of larval development and survival. The nutrients in well water are probably sufficient to support growth but not as much as in straw soak water. The longer duration of the study from instar 4 to pupae suggest that larvae experience a nutrien deficit during the pupa stage. The temperature is an important factor influencing the rate of larval development. The optimal temperature for larvae is 32^0C. The consistency in developmental times between instars in well water suggests balanced environmental conditions, although slower pupal development probably related to reduced body size or adult survival due to suboptimal nutrition.

Larval Development in Straw Soak Water

In contrast, straw soak water accelerates development across all larval stages and the transision from pupa to adult involve 0,5 days. The development more accelerated psssibly caused by the high nutrient content from organic matter in straw soak water. Nutrien-rich water supply a more abundant food source, which is known to accelerated larval development (Fahri et al. 2019; Mackay et al. 2023). The studies have indicate that larvae with access to higher nutritional level exhibit higher developmental, reflected in a 100% hatching ratio and faster life progression in straw soak water.

Egg hatching and development in tap (PDAM) water: the absence of larval development data in tap water indicates that medium doesn't support the growth of *Aedes aegypti* larvae. The research consistent with the discussion that tap water containing chemicals such as chlorine inhibits larval development. The presence of inhibitory substances can disrupt the environmental conditions necessary for larval nutrient absorption with essential physiological processes for development (Couret, Dotson, and Benedict 2014; Mackay et al. 2023). Therefore, the tap water (PDAM) doesn't support laval growth and illustrates how external factors, such as water quality and chemical content inhibit life cycle.

Comparison of Development time: Table 4 presents a comparison of the development time of *Aedes aegypti* from egg to adult in two types of water: well water and straw soak water. The data indicate that the duration from egg to larva is consistent across both media, at 2 days. However, the transition from larva to pupa is significantly longer in well

water, 4.5 days, compared to 3.5 days in straw soak water. This indicates a slower larva to pupa transition in well water. Both media exhibit the same duration of 0.5 days for the transition from pupa to adult mosquito, with no difference observed in this final phase. Consequently, the total development time from egg to adult mosquito is shorter in straw soak water (6 days) compared to well water (7 days). This suggests that straw soak water facilitates faster development of *Aedes aegypti* relative to well water, particularly due to the differences observed during the larva-to-pupa transition.

Conclusion

This paper demonstrates that different different water have significant impact to development of *Aedes aegypti* from egg to adult. Straw soak water proved to be the most supportive medium, achieving a 100% hatching rate and faster development compared to well water, which only reached a 67% hatching rate. In contrast, PDAM-treated water did not support any hatching, likely due to the presence of chemicals such as chlorine that inhibit egg and larval development. These findings highlight the potential importance of water quality, particularly its organic content, in supporting the mosquito life cycle. However, Further research is needed to thoroughly investigate the effects of different water types, the interactions between environmental factors such as temperature and humidity and the development of natural substances that can inhibit mosquito development without harming the environment. These conclusions should be supported by more detailed results and discussions, accompanied by appropriate data and references.

References

Araújo, Maisa Da Silva, Luiz Herman S. Gil, and Alexandre De Almeida E-Silva. 2012. "Larval Food Quantity Affects Development Time, Survival and Adult Biological Traits That Influence the Vectorial Capacity of Anopheles Darlingi under Laboratory Conditions." *Malaria Journal* 11: 1–9.

Arévalo-Cortés, Andrea, Yurany Granada, David Torres, and Omar Triana-Chavez. 2022. "Differential Hatching, Development, Oviposition, and Longevity Patterns among Colombian *Aedes aegypti* Populations." *Insects* 13(6).

Budiman et al. 2021. "Essential Oil as a New Tool for Larvicidal *Aedes aegypti*: A Systematic Review." *Gaceta Sanitaria* 35: S459–62. https://doi.org/10.1016/j.gaceta.2021.10.074.

Buhler, Claudia et al. 2019. "Environmental Methods for Dengue Vector Control – A Systematic Review and Meta-Analysis." *PLoS Neglected Tropical Diseases* 13(7): 1–15.

Couret, Jannelle, Ellen Dotson, and Mark Q. Benedict. 2014. "Temperature, Larval Diet, and Density Effects on Development Rate and Survival of *Aedes aegypti* (Diptera: Culicidae)." *PLoS ONE* 9(2).

Fahri, Sukmal, Susy Ariyani, Gustomo Yamistada, and Eka S. Manulang. 2019. "Hatchability of the Eggs Aedesspp in Clean and Polluted Water." *KnE Life Sciences* 2019: 134–40.

Imam, Hashmat, Ghulamuddin Sofi, Zarnigar, and Seikh Aziz. 2014. "The Basic Rules and Methods of Mosquito Rearing (*Aedes aegypti*)." *Tropical Parasitology* 4(1): 53.

Mackay, Andrew J. et al. 2023. "Larval Diet and Temperature Alter Mosquito Immunity and Development: Using Body Size and Developmental Traits to Track Carry-over Effects on Longevity." *Parasites and Vectors* 16(1): 1–13. https://doi.org/10.1186/s13071-023-06037-z.

Pradani, Firda Yanuar, Rahayu Nirmatul Mutmainah, and Sela Marlina. 2023. "Description of the Behavior of *Aedes aegypti* Mosquito Laying Eggs in Water Contaminated with Animal Feces and Soil." *Mukhtabar : Journal of Medical Laboratory Technology* 1(1): 6–13.

Soares-Pinheiro, V. C., W. Dasso-Pinheiro, J. M. Trindade-Bezerra, and W. P. Tadei. 2017. "Viabilidade de Ovos de *Aedes aegypti* (Diptera, Culicidae) Em Diferentes Condições de Armazenamento Em Manaus, Amazonas, Brasil." *Brazilian Journal of Biology* 77(2): 396–401.

Souza, Raquel Santos et al. 2019. "Microorganism-Based Larval Diets Affect Mosquito Development, Size and Nutritional Reserves in the Yellow Fever Mosquito *Aedes aegypti* (Diptera: Culicidae)." *Frontiers in Physiology* 10(APR).

Suwartawan, I. Gede Dedy et al. 2022. "Life Cycle of *Aedes aegypti* Based on Acidity Variation of Domestic Sewage Water." *Southeast Asian Journal of Tropical Medicine and Public Health* 53(Supplement 2): 348–65.

Thia Prameswarie et al. 2023. "*Aedes aegypti* Hatchability and Larval Development Based on Three Different Types of Water." *Majalah Kesehatan Indonesia* 4(1): 27–32.

Tsegaye, Arega et al. 2023. "Anopheles Larval Habitats Seasonality and Environmental Factors Affecting Larval Abundance and Distribution in Arjo-Didessa Sugar Cane Plantation, Ethiopia." *Malaria Journal* 22(1): 1–12. https://doi.org/10.1186/s12936-023-04782-1.

World Health Organization. Dengue-Global Situation. 2024. https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON51