Journal of Infrastructure, Policy and Development 2024, 8(12), 8266.
https://doi.org/10.24294/jipd.v8i12.8266

Article

Effect of water physico-chemical parameters on the development of *Aedes aegypti* mosquito larvae in endemic areas

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CITATION

Rasjid A, Suaebu S, Budirman. (2024). Effect of water physico-chemical parameters on the development of Aedes aegypti mosquito larvae in endemic areas. Journal of Infrastructure, Policy and Development. 8(12): 8266. https://doi.org/10.24294/jipd.v8i12.8266

ARTICLE INFO

Received: 29 July 2024
Accepted: 25 September 2024
Available online: 5 November 2024

Abstract: Water physico-chemical parameters, such as pH and salinity, play an important role in the larval development of *Aedes aegypti*, the primary vector of dengue fever. although the role of these two factors is known, the interaction between pH and salinity in various aquatic habitats is still not fully understood, especially in the context of endemic areas. this study explored how the interaction between pH and salinity affects the development of *Aedes aegypti* larvae in dengue hemorrhagic fever (DHF) endemic areas. this study used a pure experimental design with a posttest-only control group approach. *Aedes aegypti* instar iv larvae were obtained from eggs collected in north kolaka regency, a dhf endemic area. the independent variables tested were pH (6 and 8) and salinity (0.4 gr/L and 0.6 gr/L), with the control group using pH 7 and no salinity. a two-way anova test was used to evaluate the interaction between pH and salinity, followed by tukey's hsd post-hoc test to compare treatment groups. the results showed that, independently, pH and salinity had no significant effect on larval survival. however, the interaction between the two variables had a significant effect ($p < 0.001$). the combination of pH 8 and salinity 0.4 gr/L resulted in the highest survival rate, while pH 6 and salinity 0.6 gr/L caused a significant decrease in larval survival. the combination of alkaline pH (pH 8) and low salinity (0.4 gr/L) is the optimal condition for *Aedes aegypti* larval survival. the results of this study highlight the importance of considering the interaction between pH and salinity in environmental-based vector control strategies in endemic areas. further research is needed to explore other factors, such as aquatic microbiota and environmental variations, that may affect mosquito larval development.

Keywords: physico-chemical parameters; *Aedes aegypti*; larval development; endemic areas

1. Introduction

Approximately 4 billion people worldwide live in areas that support the transmission of diseases transmitted by *Aedes aegypti*, including dengue, chikungunya, and Zika (Brady et al., 2019; Kraemer et al., 2019). *Aedes aegypti* is the primary vector responsible for spreading these diseases, significantly impacting global public health and infecting millions of people each year (Leta et al., 2018). The mosquito's habitat varies widely, ranging from natural bodies of water such as lakes to artificial containers such as plastic containers and old tires (Mbanzulu et al., 2022; Medeiros-Sousa et al., 2015). Environmental factors, including water physicochemical parameters such as pH and salinity, play an important role in determining the ability of mosquitoes to breed and maintain their populations in endemic areas (Multini et al., 2021; Sarkar et al., 2024). In addition, rapid urbanization creates new artificial habitats that extend the risk to new areas (Wilke et al., 2017).

Overall, biotic and abiotic factors influence the life cycle of Aedes aegypti. Biotic factors such as food availability and predator presence, as well as abiotic factors such as water pH, salinity, and total dissolved solids, all play a role in influencing larval and adult mosquito development (Sivabalakrishnan et al., 2023; Torres et al., 2022). Temperature, pH, and salinity are the most significant variables influencing mosquito development from larval to adult phases (Multini et al., 2021; Sarkar et al., 2024). For example, research suggests that changes in temperature due to climate change may expand the geographic range of these vector mosquitoes, potentially increasing the risk of disease transmission in new areas (Brugueras et al., 2020; Ryan et al., 2019). Therefore, a deeper understanding of the influence of these variables is crucial, especially in mosquito control efforts in areas with a high risk of dengue transmission.

Many studies have shown the importance of the physicochemical parameters of aquatic habitats in influencing adult mosquito diversity and density. For example, variations in pH, salinity, and water temperature have been shown to affect the biological survivability of mosquitoes as well as the speed of development from larvae to adults (Medeiros-Sousa et al., 2020; Rosenfeld et al., 2019). These physicochemical characteristics also directly impact mosquitoes' potential as disease vectors, as optimal water conditions can enhance mosquito survival and production (Mbanzulu et al., 2022). In addition, water quality, including factors such as available nutrients and microorganisms in the water, can also affect larval development (Medeiros-Sousa et al., 2020).

Previous studies have shown that higher pH and certain salinity levels can slow down the rate of mosquito development. However, the specific mechanisms of these effects are not fully understood (Boerlijst et al., 2022; Kinga et al., 2022). Some studies have also shown that low salinity water conditions support *Aedes aegypti* larval development better than high salinity water (Emidi et al., 2017; Surendran et al., 2018). Therefore, it is imperative to continue in-depth research to explain how variations in these physicochemical parameters affect mosquito population dynamics (Marini et al., 2016). In addition, understanding how these factors interact with other variables, such as nutrient availability in mosquito habitats, can help improve the effectiveness of environment-based control strategies (Buxton et al., 2020; Kinga et al., 2022).

This study explores how pH and salinity affect *Aedes aegypti* larval development in natural and artificial habitats in endemic areas. By understanding these relationships, it is hoped that this research can provide better insight into the potential of environmentally-based vector control, which will support efforts to prevent the transmission of mosquito-borne diseases.

2. Materials and methods

2.1. Research design

This type of research is pure experimental research, which aims to study the effect of water physicochemical factors on the development of *Aedes aegypti* larvae. This study used a posttest-only control group design, with observations made after treatment. The research subjects were *Aedes aegypti* instar IV larvae, divided into several treatment groups based on water pH and salinity variations.

2.2. Egg survey

Entomological surveys were conducted visually from house to house and potential breeding sites. Mosquito eggs used in this study were obtained from rearing using ovitraps. Ovitraps were made from black flower pots with gauze paper as an egg-laying substrate and laid for 14 days to obtain sufficient samples.

2.3. Population and sample

The population in this study was *Aedes aegypti* larvae found in North Kolaka Regency. The sample used was 40 instar IV larvae obtained from eggs collected through ovitraps. The number of samples was selected based on treatment replication and was of sufficient size to analyze the results of the study.

2.4. Research variables

Independent Variables: pH concentration (Multini et al., 2021; Wilke et al., 2017) and salinity (0.4 gr/L and 0.6 gr/L). pH was measured using a pH meter, while salinity was measured using a salinometer. Dependent variable: The survival rate of *Aedes aegypti* larvae was calculated based on the number of larvae that survived the adult mosquito phase.

2.5. Treatment grouping

To ensure that the samples are carried out on water with a pH of 6 and 8 and for salinity, namely 0.4 gr/L and 0.6 gr/L, pH and salinity measurements are carried out; the grouping of treatments can be seen in **Tables 1** and **2**.

Table 1. pH treatment groups and their measurement methods.

Treatment 1	Measurement method
Control (pH 7)	
pH 6	pH Meter
pH 8	

Table 2. Salinity treatment groups and their measurement methods.

Treatment 2	Measurement method
Control (0 g/L salinity)	
0.4 gr/L salinity	Salinometer
0.6 gr/L salinity	

2.6. Research procedure

Eggs collected from ovitrap surveys were hatched in the laboratory. Temperature and humidity during the study were not actively controlled. However, they were measured and recorded daily using a digital thermohygrometer. Temperature and humidity during the study were within the optimal range for larval development, with temperatures ranging from 26 °C to 29 °C and relative humidity of 61%–72%. After the eggs were hatched, identification was carried out to ensure that the species obtained was *Aedes aegypti* by rearing until the larval stage. All larvae were fed the same amount of dry yeast in each treatment group to maintain nutritional consistency during observation. Each treatment consisted of variations in pH (Multini et al., 2021; Wilke et al., 2017) and salinity (0.4 gr/L and 0.6 gr/L), with 3 repetitions to ensure data accuracy. The control was conducted at pH 7 and salinity 0 g/L. Larvae were identified with a guidebook to confirm the species observed and reared until they reached the instar IV stage.

2.7. Testing method

First, instar-stage larvae obtained from rearing were tested by putting 40 larvae into water with predetermined pH and salinity variations. Each treatment was repeated three times to ensure consistency of results. Daily observations recorded larval development and survival rate until they became adults.

3. Data analysis

Data were analyzed using the Two-way ANOVA test to test whether pH and salinity significantly affected the number of eggs produced and the percentage of larval survival. They were then continued with Tukey's HSD post-hoc test to determine the comparison between groups in more detail. The significance level was set at p-value < 0.05. Analysis was performed using SPSS statistical software.

4. Results

Environmental conditions during the study showed stability, favoring the development of *Aedes aegypti* larvae. The air temperature in the research room reached 26 °C on average, with the lowest temperature being 26 °C and the highest temperature being 29 °C. Air humidity ranged from 61% to 72%, with an average humidity of 64%. These conditions are considered optimal to support larval survival, which is essential in analyzing the effect of pH and salinity on their development. Details of the environmental conditions are shown in **Table 3**.

Table 3. Average temperature and humidity measurement results.

Environmental conditions		
Time	**Temperature (°C)**	Humidity (%)
Morning (09:00 AM)	26	61
Noon (12:00 AM)	26	61
Afternoon (16:00 PM)	28	68
Average	26	64

Table 3 shows that the temperature and humidity measurements were stable across several observation times: morning, afternoon, and evening. These conditions did not significantly affect the study's results, as the temperature and humidity were within the appropriate ranges for *Aedes aegypti* larval development.

From the rearing process, 1371 mosquito eggs were obtained, with a diverse distribution of eggs depending on the pH and salinity treatments. 466 eggs were obtained in the control group, while only 11 eggs were obtained in the pH 6 group. In contrast, the pH 8 group showed the highest yield of 527 eggs. In addition, for salinity variations of 0.4 gr/L and 0.6 gr/L, 192 and 175 eggs were obtained, respectively. These results indicate that the pH 8 condition is more optimal for mosquito oviposition activity than the pH 6 and salinity variation groups (**Table 4**). By repeating the observation process up to 3 times, the highest number of mosquito eggs was obtained at pH 8 with a total of 572 eggs, with an average total eggs obtained of 66 eggs/day (for 3 weeks).

Table 4. Results of ovitrap installation of *Aedes aegypti* eggs for 8 days based on pH.

Treatment	Replication	Time Period								
		H1	H2	H3	H4	H5	H6	H7	H8	Total
	I	21	17	15	12	24	13	19	20	141
Control	II	19	22	26	18	13	21	17	28	164
	III	15	21	29	22	16	21	20	17	161
Average		18.3	20	23.3	17.3	17.6	18.3	18.6	21.6	466
	I	3	0	0	2	1	0	0	0	6
pH 6	II	0	0	1	0	3	0	0	0	4
	III	0	0	0	0	1	0	0	0	1
Average		1	0	0.3	1.5	1.6	0	0	0	11
	I	27	15	25	11	21	28	18	28	173
pH 8	II	24	18	28	25	22	21	21	19	178
	III	21	24	26	22	19	19	28	17	176
Average		72	57	79	58	62	68	67	64	**527**

Table 5. Ovitrap installation results of *Aedes aegypti* eggs for 8 days based on salinity.

Treatment	Replication	Time Period								
		H1	H2	H3	H4	H5	H6	H7	H8	Total
	I	21	17	15	12	24	13	19	20	141
Control	II	19	22	26	18	13	21	17	28	164
	III	15	21	29	22	16	21	20	17	161
Average		18.3	20	23.3	17.3	17.6	18.3	18.6	21.6	466
	I	7	5	11	9	5	5	12	2	56
0.4 gr/L salinity	II	9	11	9	5	13	3	10	9	69
	III	3	13	7	5	12	11	9	7	67
Average		19	29	27	19	30	19	31	18	192
	I	9	6	7	8	4	6	3	3	46
Salinity 0.6 gr/L	II	14	11	9	6	10	11	4	7	72
	III	5	13	10	14	7	5	3	0	57
Average		28	30	26	28	21	22	10	10	175

The number of eggs obtained during the 3 repetitions was then hatching at the same pH and hatched as much as 46.53% with a total of 638 eggs hatched. At the same time, those that became adult mosquitoes were 21.95% of the total eggs, with only 301 larvae to adult mosquitoes that could evolve. At pH 6, the lowest result was 11 eggs, with an average of 0–1.6 eggs. The eggs that have been obtained are then collected and hatched in the same water, namely control water, and transferred to each sample water until they become adult mosquitoes, as shown in **Tables 4** and **5**.

Table 6. Results of instar I to instar IV larval development based on pH.

Treatment	**Replication**	**Time period**							
		H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**
Control	I	40	40	40	40	40	39	40	40
	II	40	40	40	40	40	40	39	40
	III	40	40	40	40	40	40	40	39
Percentage of life		100%	100%	100%	100%	100%	99.1%	99.1%	99.1%
pH 6	I	37	28	13	8	0	0	0	0
	II	34	25	15	6	1	0	0	0
	III	31	21	15	4	0	0	0	0
Percentage of life		85%	61.6%	35.8%	15%	0.8%	0%	0%	0%
pH 8	I	40	40	35	31	31	28	28	28
	II	40	38	38	35	32	31	31	29
	III	40	40	36	32	29	29	28	27
Percentage of life		100%	98.3%	90.8%	81.6%	76.6%	73.3%	72.5%	70%

Table 7. Developmental results of instar I to instar IV larvae based on salinity.

Treatment	**Replication**	**Time period**							
		H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**
Control	I	40	40	40	40	40	39	40	40
	II	40	40	40	40	40	40	39	40
	III	40	40	40	40	40	40	40	39
Percentage of life		100%	100%	100 %	100%	100%	99.1%	99.1%	99.1%
0.4gr/L salinity	I	37	35	31	29	25	25	22	20
	II	39	31	29	25	23	23	20	19
	III	37	33	27	25	22	21	19	17
Percentage of life		94%	82.5 %	72.5 %	65.8%	58.3%	57.5%	50.8%	46.6%
Salinity 0.6 gr/L	I	37	36	36	31	29	23	14	11
	II	34	31	31	29	29	24	18	14
	III	38	35	35	34	30	29	22	17
Percentage of life		90.8%	85%	85%	78.3%	73.3%	63.3%	45%	35%

Based on the observations, pH and salinity variations significantly affected the survival of *Aedes aegypti* larvae. As shown in **Table 6**, in the control group (pH 7), almost all larvae survived with a 100% survival percentage until the fourth day and slightly decreased to 99.1% on the eighth day. The pH 6 group showed a drastic decrease, with a survival percentage of 85% on the first day and reaching 0% on the sixth day. In contrast, survival in the pH 8 group remained high, with a percentage of 100% on the first day, and decreased gradually to 70% on the eighth day.

Table 7 shows that the control group without salinity also showed optimal survival, reaching 100% on the first to the fourth day and decreasing slightly to 99.1% on the eighth day. Larval survival at 0.4 gr/L salinity decreased from 94% on day one to 46.6% on day eight, while at 0.6 gr/L salinity, a steeper decline occurred, from 90.8% on day one to only 35% on day eight.

Figure 1 visualizes the survival trend based on pH variation. The control group (pH 7) showed high stability throughout the observation, while pH 6 experienced a significant decrease after the first day. At pH 8, the decline was gradual, with survival remaining high until the end of the observation. **Figure 2** shows that without salinity, survival remained stable, while at salinities of 0.4 gr/L and 0.6 gr/L, there was a sharper decline in survival over time, with the most drastic decline at 0.6 gr/L salinity.

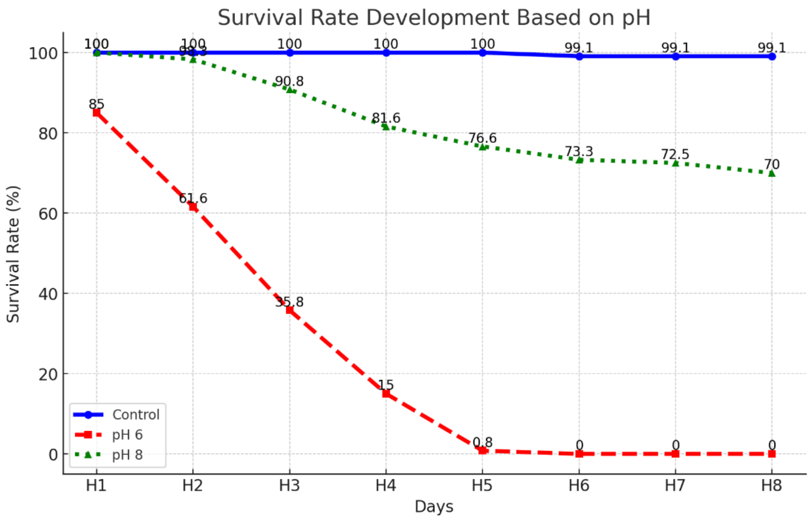

Figure 1. Survival rate of *Aedes aegypti* larvae based on pH.

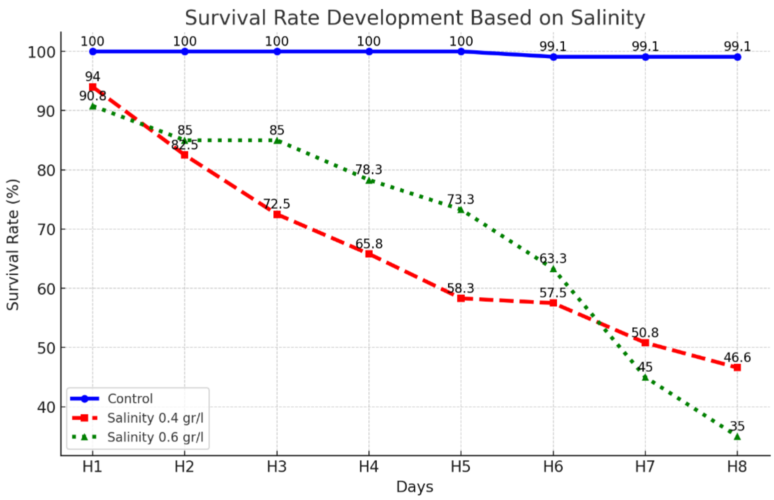

Figure 2. Survival rate of *Aedes aegypti* larvae by salinity.

A two-way ANOVA test was used to evaluate the effect of pH (pH 6, pH 8, and control) and salinity (0.4 gr/L, 0.6 gr/L, and control) as well as the interaction between the two factors on the percentage survival of *Aedes aegypti* larvae (**Table 8**). The analysis showed that, individually, neither pH nor salinity significantly affected larval survival. The results of the analysis for the pH variable showed a value of $F = -1.36 \times 10^{-13}$ and $p = 1.00$, indicating that variations in pH did not cause significant differences in larval survival. Similarly, $F = 0.048$ and $p = 0.828$ were obtained for the salinity variable, indicating that variation in salinity levels also did not have a significant effect when analyzed separately.

Table 8. Two-way ANOVA statistical test results.

Source	Sum of squares	df	Mean square	F	Sig.
pH	−1.0352E-10	2	−5.17601E−11	−1.36103E−13	1
Salinity	36.41008	2	18.20504	0.04787014	0.8280826
pH * Salinity	33011.86	4	8252.965	21.70116	4.06628E−08
Residuals	13310.52	35	380.3005714		

Note: An asterisk (*) indicates an interaction effect between pH and salinity in the statistical analysis.

Table 9. Tukey's HSD post-hoc statistical test results.

Group 1	Group 2	Mean difference (I-J)	Std. Error	Sig. (*p*-value)	Lower bound	Upper bound	Reject null hypothesis
control & 0.4 gr/L	control & 0.4 gr/L	control & 0.4 gr/L		control & control	control & control	control & control	pH 6 & 0.6 gr/L
control & control	pH 6 & 0.6 gr/L	pH 6 & control		pH 6 & 0.6 gr/L	pH 6 & control	pH 8 & control	pH 6 & control
33.6625	3.4625	−41.225		−30.2	−74.8875	−16.775	−44.6875
0.012	0.9964	0.0014		0.0295	0.0	0.4351	0.0005
5.6288	−24.5712	−69.2587		−58.2337	−102.9212	−44.8087	−72.7212
61.6962	31.4962	−13.1913		−2.1663	−46.8538	11.2587	−16.6538
True	False	True		True	True	False	True

However, the interaction between pH and salinity significantly affected larval survival. The values of $F = 21.70$ and $p < 0.001$ indicated a combined effect of pH and salinity on larval survival, which was not seen when the two variables were analyzed independently. This interaction suggests that pH's effect on larval survival depends on the salinity level and vice versa. Tukey's HSD post-hoc test was conducted to evaluate the differences between groups further (**Table 9**). The results showed that there was a significant difference between the control group (pH 7 and no salinity) and the group that received 0.4 gr/L salinity treatment. The *p*-value = 0.012, and the mean difference of 33.66 indicated that larval survival was higher in the group with 0.4 gr/L salinity than in the control group. In addition, a significant difference was also found between the control group and the group with pH 6, with a *p*-value = 0.0014 and a mean difference of −41.22. It is shown that the larvae in the pH 6 condition had a significantly lower survival rate compared to the control group.

No significant differences were found in some other comparisons, such as between the group with pH 6 and salinity 0.6 gr/L and the other groups, where the *p*-

value was more than 0.05. These indicate that certain combinations of pH and salinity do not always result in significant differences in larval survival.

5. Discussion

The development of *Aedes aegypti* mosquito larvae is strongly influenced by the physico-chemical conditions of water, particularly pH and salinity, which significantly determine their survival. This study found that pH 8 resulted in the highest survival rate, while pH 6 caused a significant decline, reaching 0% on the sixth day. This finding is in line with previous studies showing that *Aedes aegypti* thrive optimally in water conditions with neutral to alkaline pH (Hai et al., 2021a; Tirado et al., 2017). Acidic pH conditions, such as pH 6, have been shown to be unfavorable for larval development (Tirado et al., 2017; Torres et al., 2022). A study also found that *Aedes aegypti* can survive in water with pH close to 8.3 and salinity up to 7.9 parts per thousand (ppt) (Hai et al., 2021b).

Two-way ANOVA test results showed that the interaction between pH and salinity had a significant effect on larval development, with $F = 21.70$ and $p < 0.001$. This indicates that these factors cannot be analyzed separately, but rather have a significant combined effect (Mbanzulu et al., 2022; Sarkar et al., 2024). Individually, the pH variation alone did not yield significant results with a p value = 1.00, nor did salinity ($p = 0.828$). However, when these two variables were interacted, the results showed a significant effect on larval survival (Sivabalakrishnan et al., 2023).

On further analysis through Tukey's HSD post-hoc test, it was shown that significant differences occurred between larval groups reared at pH 8 and pH 6. At pH 6, larvae could not survive until the sixth day, while at pH 8, survival remained high until the eighth day. In addition, the 0.4 gr/L salinity group also showed higher survival rates than the 0.6 gr/L salinity group (Akhter et al., 2017; Medeiros-Sousa et al., 2020; Putri et al., 2023). These results provide strong evidence that more alkaline and lower salinity water conditions are more favorable for *Aedes aegypti* larval development, which could be an important factor in mosquito habitat management in endemic areas (Donini et al., 2007; Ratnasari et al., 2020).

Salinity also plays an important role in the survival of *Aedes aegypti* larvae. The results of this study showed that a salinity of 0.4 gr/L was more optimal than 0.6 gr/L, with a higher survival rate. At 0.6 gr/L salinity, there was a drastic decrease in survival to 35% on the eighth day. Salinity of 0.4 gr/L was found to be a more optimal condition than 0.6 gr/L, in accordance with research by Emidi et al. (2017) which showed that *Aedes aegypti* larvae prefer low salinity (Emidi et al., 2017). Donini et al. (2007) also supported these findings, showing that larval survival is better in environments with low to moderate salinity. Other studies have shown that larvae reared in higher salinity water conditions exhibit physiological changes in ion uptake mechanisms and morphological adjustments in the anal papillae to adapt to different environments (Donini et al., 2007; Ramasamy et al., 2021).

This study provides a new contribution to the literature on the influence of physico-chemical factors on the development of *Aedes aegypti* larvae in natural and artificial habitats. The results of this study indicate that pH 8 with low salinity is the optimal condition for larval development, in line with research by Tirado et al. (2017)

who found that *Aedes aegypti* larvae develop optimally at alkaline pH (Tirado et al., 2017). This is also supported by the findings of Ratnasari et al. (2021), where pH 8 provides the most optimal conditions for larval survival, although larvae can still develop at lower pH (Ratnasari et al., 2021). In addition, changes in the physiological adaptation of larvae in high salinity environments show that larvae can survive in saltier water conditions, but with a consequent decrease in ion uptake efficiency (Akhter et al., 2017; D'Silva et al., 2017).

In addition to pH and salinity, stable water quality, especially temperature, is also an important factor in supporting larval survival. Laboratory temperatures ranging from 26 °C to 29 °C during the study were considered optimal for larval development (Bayoh and Lindsay, 2004). These temperature conditions support the results found, where the highest survival occurred under alkaline pH and low salinity conditions.

When compared with recent literature, this study supports some important findings. Recent research by Ratnasari et al. (2021) showed that *Aedes aegypti* also thrive optimally at neutral to alkaline pH, which is similar to the results of this study where pH 8 resulted in the highest survival (Ratnasari et al., 2021). However, differences were found in Ratnasari's study which recorded larval survival at higher salinity conditions (>1 g/L), while this study showed a significant decrease at 0.6 gr/L salinity.

Although this study provides important insights into the effects of pH and salinity on *Aedes aegypti* larval development, there are several limitations that need to be noted. First, this study was conducted under controlled laboratory conditions, so the results may not fully reflect natural conditions in the field (Thia Prameswarie et al., 2023; Tirado et al., 2017). Other factors, such as the presence of natural predators and the composition of nutrients in the water, may affect results in more complex environments (Bellamy et al., 2024; Levi et al., 2014; Medeiros-Sousa et al., 2015). Secondly, this study did not measure the influence of other variables such as the microbiota in the water, which may also play a role in larval survival (Sarkar et al., 2024; van Schoor et al., 2020). Thirdly, this study only included two variations of salinity and pH, so the influence of other wider variations has not been fully revealed (Reiskind and Janairo, 2018). For future research, further exploration of these variables, as well as interactions with other environmental factors, may provide greater insight into more effective mosquito control efforts (Putri et al., 2023; Souza et al., 2019).

6. Conclusions

This study showed that the interaction between pH and salinity significantly affected the survival of *Aedes aegypti* larvae. The combination of pH 8 and salinity 0.4 gr/L resulted in the highest survival, while pH 6 and salinity 0.6 gr/L drastically reduced survival. These findings emphasize the importance of considering the interaction of environmental factors in mosquito control strategies in endemic areas. Although the study was conducted under laboratory conditions, these results provide important insights into how pH and salinity affect larval development. Further research is needed to explore other factors, such as microbiota and natural environmental variations, that might affect mosquito survival in their natural habitat.

Author contributions: Conceptualization, AR and SS; methodology, AR and SS; software, B; validation, AR, SS and B; formal analysis, SS and B; investigation, AR and SS; resources, AR; data curation, AR and SS; writing—original draft preparation, B; writing—review and editing, B; visualization, B; supervision, AR and SS; project administration, SS; funding acquisition, AR. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This study was conducted in North Kolaka, Indonesia, a dengue fever area, by a team from the Makassar Health Polytechnic.

Conflict of interest: The authors declare no conflict of interest.

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