

# Salinity tolerance of larvae *Aedes aegypti* inland and coastal habitats in Pasangkayu, West Sulawesi, Indonesia

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**Abstract.** Ratnasari A, Jabal AR, Syahribulan, Idris I, Rahma N, Rustam SNRN, Karmila M, Hasan H, Wahid I. 2021. Salinity tolerance of larvae *Aedes aegypti* inland and coastal habitats in Pasangkayu, West Sulawesi, Indonesia. *Biodiversitas* 22: 1203-1210. *Aedes aegypti* L. is the primary arboviral vector generally found in freshwater, but it is also observed in brackish water. The study analyzes habitat characteristics, larvae adaptation, and oviposition preference in salinity levels over the coastal and inland ecosystem in Pasangkayu District of West Sulawesi. The larvae were reared until adults and have larval of first progeny were examined the salinity tolerance in five salinity levels. Ovitrap-based experiments were carried out in the laboratory using adult *Ae. aegypti* colonies from inland and coastal areas. Eggs on filter paper (ovitrap) were identified and counted. Data were analyzed using LC50 (larvae with salinity tolerance) and Pearson correlation (correlation between the larvae phase two ecosystem factors). Total larvae observed in coastal areas and inland are 1437 and 1288, respectively. The salinity tolerance test showed that Instar IV is highly adapted to the saline environment. The larvae from the coastal (inland) area can tolerate salinity up to 13 (10) ppt. Mosquitoes can lay many eggs in 3 ppt salinity: 27.1% and 20.8% for coastal and inland colonies, respectively. Pearson correlation analysis showed a significant correlation between the larval instar stage of *Ae. aegypti* from coastal and inland ecosystems with the ability to survive at the salinity level ( $p < 0.01$ ). This study is expected to be a source of information on the adaptation of *Ae. aegypti* mosquito to salinity in coastal and inland ecosystems. The findings can be considered in vector control efforts on brackish water habitats.

**Keywords:** *Aedes aegypti*, coastal, inland, Pasangkayu, salinity

## INTRODUCTION

Arboviral diseases have been widely reported in the world (Soni et al. 2020). The vector that causes the disease (dengue, yellow fever, and chikungunya) in humans over the tropical and sub-tropical countries is *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae) (Ramasamy et al. 2011; Augustina et al. 2020). Globally, there are 3.97 billion people in 128 countries at risk of this disease. Dengue vector breeding sites are not given much attention in coastal areas. Brackish water habitats are a neglected source of dengue fever vectors on tropical beaches in the world (Ramasamy and Surendran 2012).

*Aedes aegypti* larvae found in a used drink containing brackish water in coastal areas with a salinity of 2-14 ppt and in wells with a salinity of 2-9 ppt (Surendran et al. 2012). Results of a laboratory study on *Ae. aegypti* from coastal Thirunelvely tolerated 18 ppt salinity and in inland Batticaloa adapted to salinity of 14 ppt, in Sri Lanka (Ramasamy et al. 2011). Results of a laboratory study on *Ae. aegypti* from coastal tolerated 18 ppt salinity and in inland adapted to salinity of 12 ppt, in Jaffna District, Sri Lanka (Ramasamy et al. 2011). In Brunei Darussalam, *Ae.*

*aegypti* from coastal tolerated 10 ppt salinity and in inland adapted to salinity of 8 ppt (Idris et al. 2013).

The information on the adaptability of *Ae. aegypti* to salinity in coastal and inland areas, especially in the Pasangkayu District (Formerly Mamuju Utara District), West Sulawesi Province, Indonesia is essential to know the salinity level to control the spread of the dengue vector. Bambalamotu (i.e. Randomayang coastal area) and Martajaya Sub-districts are endemic areas of DHF every year (West Sulawesi Health Office 2020). DHF cases continue to increase in West Sulawesi every year, and the highest is in Pasangkayu District with two sub-districts of Bambalamotu and Martajaya. This study aimed to analyze the habitat characteristics, larvae adaptation, and oviposition preference in salinity levels. This research is expected as a source of information on the salinity adaptability of *Ae. aegypti* in two different ecosystems in Pasangkayu District.

## MATERIALS AND METHODS

This experimental study tested the salinity tolerance of *Ae. aegypti* from coastal Randomayang and inland Martajaya, Pasangkayu District, West Sulawesi, Indonesia.

### Study area

Larvae were collected on the coast of Pasangkayu District from January to August 2020. The coastal sampling location is at coastal Randomayang, Bambalamotu Sub-district, Pasangkayu District, West Sulawesi (-1°03'46.9" S, 119°27'58.8"E). The inland area covers the Martajaya Sub-district of Pasangkayu District, West Sulawesi (-1°10'23.9"S, 119°24'52.8"E) with the consideration that the location is located on the outskirts of the city center with easy access and densely populated (Figure 1).

### Larval collection

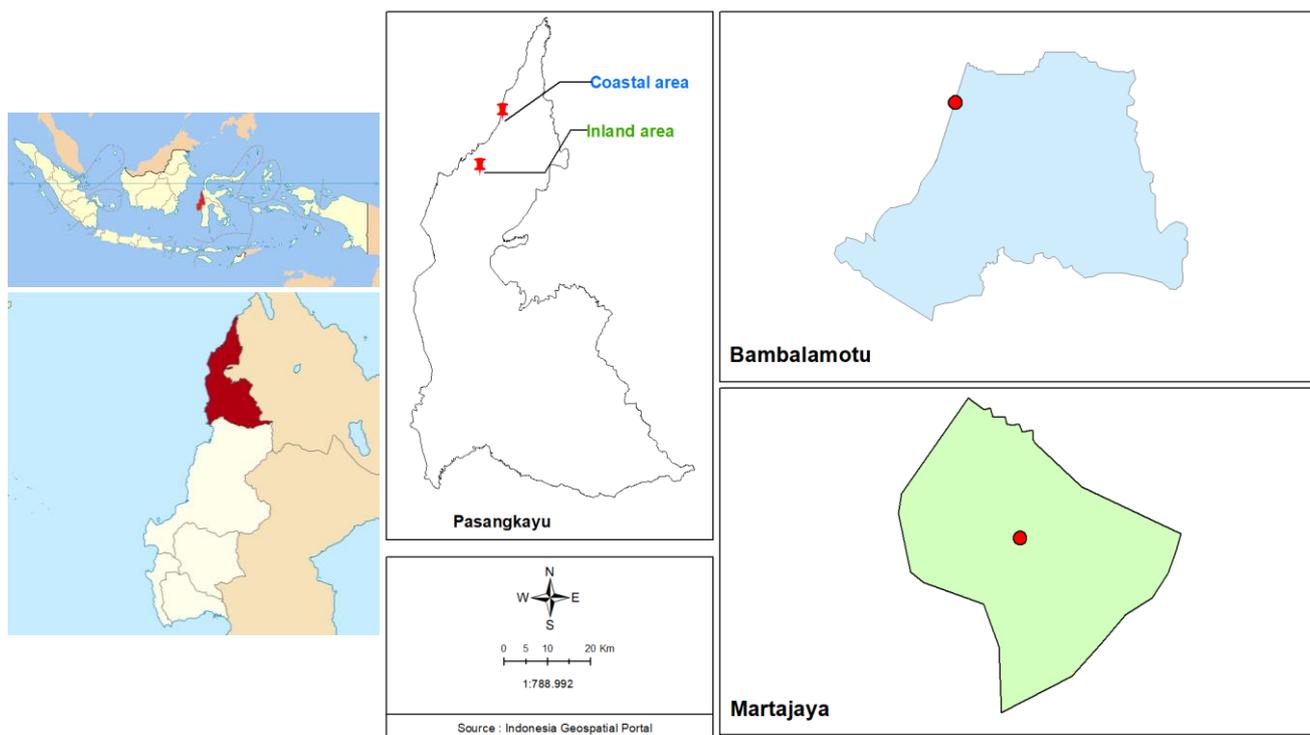
Sampling was collected by searching for *Ae. aegypti* breeding habitat in inland and coastal area. All larvae were collected using a Pasteur pipette. The water salinity of larvae habitat was used with a refractometer and salt meter (Lutron YK-31SA). The instrument was calibrated with a solution of 0 to 30 ppt sodium chloride in the laboratory, and the measurement of water pH was done by pH meter. All larvae with water in the containers were transferred to the sample bottles and labeled, then the distance of the sample coordinates from the coastline used GPS (Garmin Montana 680). All samples were brought to the Entomology Laboratory of Faculty Medicine, Hasanuddin University, Makassar, Indonesia.

### Mosquito identification

The identified larvae were positioned on glass objects by a needle. The observation was conducted with a stereomicroscope (Nikon SMZ745) with 10x-40x magnification by a taxonomic identification key of *Aedes* larvae, refer to Ministry of Health, Indonesia (2017).

### Tolerance test

*Aedes. aegypti* larvae collected from coast were reared by using seawater taken in their natural habitat and *Ae. aegypti* larvae from inland were reared by using freshwater. Larvae were fed with fish pellets twice a day. Larvae were reared until adults using separate sterile cages in a laboratory with temperature 28°C and humidity 60-80%. Female adult *Ae. aegypti* were fed with blood mice and male adults fed sugar water. The result mated of first progeny, larvae were used for the salinity tolerance test. The salinity tolerance test was done on Instars I, II, III, and IV. Dilution of seawater made by mixing seawater with freshwater to form salinity of 0, 3, 5, 10, 15, and 20 ppt. Measurement of salinity used a salt meter (Lutron YK-31SA) to determined salinity levels. Ten larvae for each instar I-IV were put in a plastic container filled with saline water at levels 0, 3,5,10,15, and 20 ppt. Furthermore, it was closed using plastic to avoid evaporation. The test was repeated three times in parallel for each salinity level. The number of larvae *Ae. aegypti* that succeeded in becoming adult mosquitoes recorded for each level of salinity.



**Figure 1.** Two sampling locations (red marker): Bambalamotu Sub-district for coastal area and Martajaya Sub-district for inland area of West Sulawesi Province, Indonesia

### Oviposition preference for fresh and brackish water in laboratory

A total of 60 adult mosquitoes (30 females and 30 males) were put in each of six mosquito cages. Water with salinity levels of 0,3,5,10,15 and 20 ppt was put into each plastic container in each cage. Filter paper used as an ovitrap was put in the container. Counting mosquito eggs trapped in ovitrap was conducted every week and was done for four weeks (Surendran et al. 2007).

### Ethics

This research did approve by the Health Research Ethics Committee of the Hasanuddin University Medical Faculty with the attached number 558/UN4.6.4.5.31/PP36/2020.

### Data analyze

The distribution of *Ae. aegypti* larvae mapped using ArcGIS geographic information system software version 10.5. Habitat characteristics and percentage of larvae from coastal and inland analyzed chi-square analysis. Larval survival and salinity tolerance used probit analysis and ratio test (LC50) with a confidence limit of 95% (Wheeler et al. 2006). The correlation between larval stages from two habitat ecosystems analyzed Pearson correlation IBM SPSS version 24.

## RESULTS AND DISCUSSION

### Larval distribution

Distribution of *Ae. aegypti* in the coastal area was 52.7% (n = 1437) and inland was 47.3% (n = 1288). The larval habitat characteristics in the two ecosystems showed the highest acidity level in the drum water species (Table

1). The distribution of larvae visually is shown in Figure 2. Survival and salinity tolerance of *Ae. aegypti* in the coastal Randomayang and the inland Martajaya in the Pasangkayu district are presented graphically (Figure 3) with the relevant parameters tabulated (Table 2).

### Survival larva *Ae. aegypti* in coastal and inland habitats

The larval survival curves in the two ecosystems differ along the gradient of the salinity assay. Both populations (Coastal and Inland) had a 100% survival rate in controls (0 ppt). Instar III and IV larvae survived at 3 and 5 ppt salinity in more than 90% of coastal ecosystems. A sharp reduction of about 50% of the observed survival rate occurred at 15 ppt salinity, and total mortality occurred at 20 ppt salinity. Instar III and IV larvae survived in 3 and 5 ppt salinity in more than 80% inland ecosystem. A sharp reduction of about 50% of the observed survival rate occurred at 10 ppt salinity, and total mortality occurred at 20 ppt salinity (Figure 3).

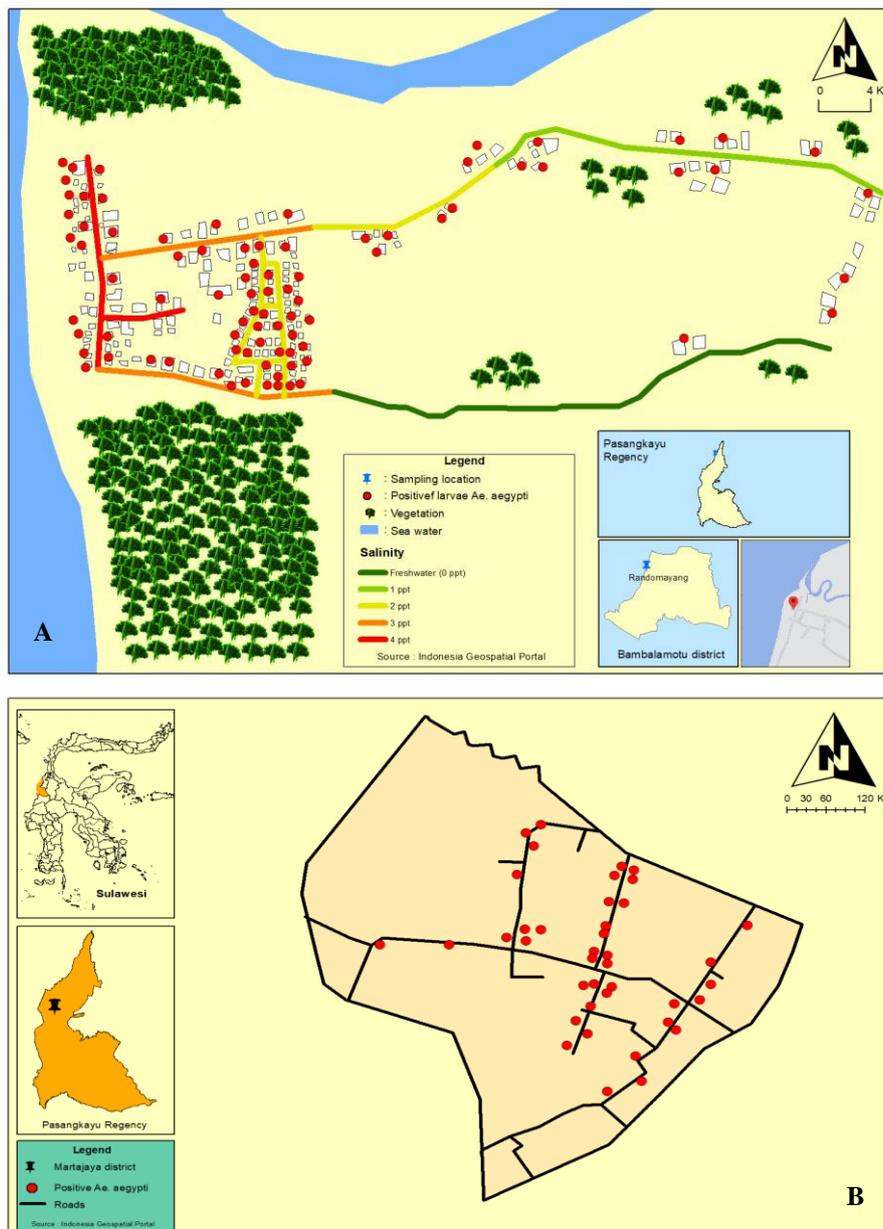
**Table 2.** Salinity tolerance of *Ae. aegypti* larvae in the laboratory

Ecosystems	Larva <i>Ae. aegypti</i> stage	Maximum salinity tolerance <i>Ae. aegypti</i> larvae for 100% adulthood in salinity	LC <sub>50</sub> ppt (CI)
Coastal	1 <sup>st</sup>	8	10.9 (10-11,8)
	2 <sup>nd</sup>	8	10.8 (9.9-11.7)
	3 <sup>rd</sup>	10	12.6 (11.7-13.5)
	4 <sup>th</sup>	10	13.1 (12.1-14.0)
Inland	1 <sup>st</sup>	5	9.5 (8.6-10.4)
	2 <sup>nd</sup>	7	9.7 (8.8-10.7)
	3 <sup>rd</sup>	8	10.0 (9.3-11.0)
	4 <sup>th</sup>	9	10.8 (9.9-11.6)

Note: LC<sub>50</sub> test with CI – 95% confidence interval

**Table 1.** Distribution and Characteristic habitat larva *Ae. aegypti* in coastal and inland area

Region	Container type	Containers inspected	Positive container	Positive %	pH	Salinity (ppt)	Number of larva	% Larva
Randomayang	Coconut shell	5	2	40	5.9	4	8	0.3
	Wooden boat	10	6	60	5.9	4	189	6.9
	Well	10	3	30	6.4	2	144	5.3
	Plastic glass	5	2	40	5.9	4	21	0.8
	Discarded buckets	20	6	30	6	3	247	9.1
	Drums	20	10	50	6.7	1	410	15.0
	Flower pot	10	5	50	7	0	130	4.8
	Discarded tires	10	4	40	7	0	248	9.1
	Discarded cans	5	1	20	6	3	10	0.4
	Concrete tank	5	1	20	6.7	1	30	1.1
		<b>100</b>	<b>40</b>	<b>40</b>	<b>6.4</b>	<b>2.2</b>	<b>1437</b>	<b>52.7</b>
Martajaya	Bathtub	10	4	40	7	0	168	6.2
	Used buckets	10	10	20	6.9	0	80	2.9
	Used tires	15	15	26,67	7	0	199	7.3
	Flower pot	15	15	46,67	7	0	154	5.7
	Used cans	10	10	60	7	0	136	5.0
	Trash can	15	15	53,33	7	0	157	5.8
	Cans cat	15	15	46,67	6.5	0	133	4.9
	Drum water	10	10	60	7	0	261	9.6
			<b>100</b>	<b>94</b>	<b>44.17</b>	<b>6.9</b>	<b>0</b>	<b>1288</b>



**Figure 2.** A. Distribution map of *Ae. aegypti* in coastal area, B. Distribution of *Ae. aegypti* in inland area of Pasangkayu District, West Sulawesi, Indonesia

### Salinity tolerance of *Ae. aegypti* larvae

Tolerance of *Ae. aegypti* larvae to salinity from the LC<sub>50</sub> test showed differences in each instar stage. Instar IV is highly adapted to salinity to become adult mosquitoes. Two ecosystems show that coastal areas are better able to tolerate salinity up to 13 ppt while inland can only tolerate salinity up to 10 ppt (Table 2).

### Adaptability to lay eggs in various salinity levels

*Aedes. aegypti* usually lay their eggs in freshwater, but these mosquitoes are adapted to different environments and can be a source of vector transmission that is difficult to control. *Ae. aegypti* in two ecosystems treated on the media

to lay eggs with the water's salinity levels showed a significant difference. Colony *Ae. aegypti* was able to lay many eggs in coastal areas, in contrast to inland colonies, which show fewer eggs at each salinity level. At the testing stage, mosquitoes can lay eggs at the highest salinity level (3 ppt), with a percentage of 27.1% for coastal colonies and 20.8% for inland colonies (Table 3).

### Correlation of survival and salinity of larvae *Ae. aegypti*

Pearson correlation analysis showed significance at the larval instar stage of *Ae. aegypti* of both ecosystems with the ability to survive at the salinity level ( $p = 0.000$ ) (Table 4).

**Table 3.** Characteristics ovitraps of *Ae. aegypti* oviposition in laboratory

Salinity (ppt)	1 <sup>st</sup> week		2 <sup>nd</sup> week		3 <sup>rd</sup> week		4 <sup>th</sup> week		Percent of total	
	R	M	R	M	R	M	R	M	Coastal	Inland
0	211	210	161	170	115	84	78	53	40.3	59.0
3	120	80	100	50	98	33	62	19	27.1	20.8
5	90	36	50	40	41	21	26	13	14.8	12.5
10	81	25	52	27	38	7	15	0	13.3	6.7
15	29	0	15	0	9	0	4	0	4.1	0
20	6	0	0	0	0	0	0	0	0.4	0

Note: Ecosystem R: Randomayang colonies, M: Martajaya colonies

**Table 4.** Correlation of survival and salinity in each *Ae. aegypti* larvae instar in ecosystems

Instar	Correlation salinity	Survive	
		Coastal	Inland
1 <sup>st</sup>	Pearson Correlation	-.888**	-.937**
	Sig. (2-tailed)	0.000	0.000
2 <sup>nd</sup>	Pearson Correlation	-.901**	-.922**
	Sig. (2-tailed)	0.000	0.000
3 <sup>rd</sup>	Pearson Correlation	-.912**	-.921**
	Sig. (2-tailed)	0.000	0.000
4 <sup>th</sup>	Pearson Correlation	-.918**	-.861**
	Sig. (2-tailed)	0.000	0.000

Note: \*\*. Correlation is significant at the 0.01 level (2-tailed)

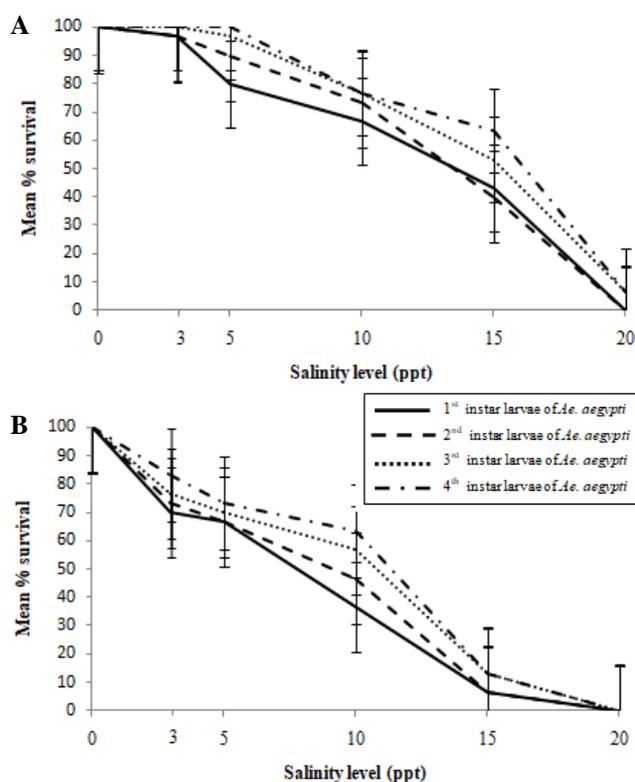
## Discussion

Dengue fever is an acute febrile illness caused by the dengue virus (DENV). *Ae. aegypti* is the primary vector of dengue fever, chikungunya, yellow fever, and the Zika virus worldwide. Mosquito *Ae. aegypti* can transovarially reduce the virus in mosquito heredity, especially in females (Le Coupanec et al. 2017; Soni et al. 2020; Souza et al. 2019). Indonesia is a tropical country that is very supportive of *Ae. aegypti* (Sasmono et al. 2015). The number of dengue hemorrhagic fever (DHF) in 2020 was 95.89 cases in Indonesia. The spread of dengue was fast, and mosquitoes were difficult to control (Ministry of Health, Republic of Indonesia 2020). Dengue fever in the West Sulawesi region in 2020 was 614 cases.

Distribution of larvae *Ae. aegypti* (n=2752) was found in two locations and the highest in coastal Randomayang (n=1437). The highest percentage of larvae from the two habitats was in the type of container drum water, but the difference in the coastal is the water contains salinity in the container. Drum water was mostly found because people use it to collect water. Lack of clean water capacity is the reason residents use water drums as water reservoirs. Water drums were breeding places for *Ae. aegypti* in developing countries (Hemme et al. 2009; Ferde et al. 2018). Other studies reported the percentages of water drums being used as a breeding site for *Ae. aegypti* 19.3% (Midega et al. 2006), 19.7% (Ngugi et al. 2017), and 31.8% (Tedjou et al. 2018).

Chemical characteristic of larval habitat is classified based on the salt content. Freshwater habitats have a salinity of <0.5 ppt, brackish water 5 to 30 ppt, seawater 30 to 50 ppt, and brine water which is very rich in dissolved salts has a salinity > 50 ppt (Lincoln et al. 1982). The coastal brackish and salty habitats consist of salt marshes, mangrove swamps, rock pools, and lakes. The littoral habitat zone has an ion ratio similar to that of seawater. The salinity of the brackish and salty habitats tends to fluctuate widely, decreasing due to rain or exposure to freshwater, and increases by evaporation. Sea level rise due to global warming could result from the expansion of naturally brackish surface water bodies in coastal areas and a further effect, namely the level of water withdrawal from freshwater aquifers in coastal areas by expanding the population of Nicholls et al. (2007). These conditions can lead to mosquito species emergence over a long period with different salinity suitability (Mosha and Muteru 1982).

Even though most mosquito larvae live in freshwater, there was 5% of species live in brackish or saltwater, such

**Figure 3.** Survival larvae of *Ae. aegypti* in coastal (A) and inland (B)

as the genera *Aedes*, *Opifex*, *Prophora*, *Aedomyia*, *Culex*, *Uranotaenia*, and *Anopheles* (O'Meara 1976). Various *Aedes* genera, such as *Ae. togoi* found in coastal marshes. Sea level rise can increase the abundance of mosquito vectors that can adapt to saline. *An. giles* was the first vector of malaria in Sri Lanka. Its freshwater habitat was adapted to undergo preimaginal development in brackish waters with 4 ppt salinity (Surendran and Ramasamy 2010). Besides, *An. subpictus* and *An. sudaicus* tolerated various salinities (euryhaline) in Sri Lanka. The larvae were isolated from freshwater with 30 ppt water in the lagoon (Surendran et al. 2011).

*Aedes. aegypti* are generally only considered to reproduce only in freshwater, while mosquitoes can adapt to various environments (Rueda 2008). *Ae. aegypti* was found to adapt to coastal habitats close to human settlements (Ratnasari et al. 2020). The adaptation process of *Ae. aegypti* in saline habitat occurred in coastal areas because of the mixture of freshwater with seawater (Jude et al. 2010). The salinity tolerance of *Ae. aegypti* in coastal areas is an adaptive response to the extensive use of insecticides for agriculture in the inland area (Ramasamy and Surendran 2011).

In this study, larvae *Ae. aegypti* was not able to survive with a salinity of 20 ppt from the coastal and inland in the laboratory. Decreased survival was rapid at 10 and 15 ppt and 20% survival at 20 ppt salinity. The adaptability of *Ae. aegypti* at 90% at 3.5 ppt salinity, and 0% at 17.5 ppt salinity (Clark et al. 2004). According to Ramasamy et al. (2014), larva *Ae. aegypti* from the coastal area survived with a salinity of 18 ppt in the second generation of these mosquitoes. In the fifth generation, the mosquitoes adapted at a salinity of 20 ppt. The ability limit for *Ae. aegypti* tolerates a salinity of 17.5 ppt (de Brito Arduino et al. 2015). The container sample contained saline content in most sampling locations except at a distance of more than 1 km from the coastline. On laboratory tests, the IV instar can tolerate salinity up to a salinity of 15 ppt.

Mosquito larvae in brackish waters can live for 24 hours at 1000 mOsm, but cannot mature unless salinity was lowered (Wigglesworth 1933; Bradley 1987). The osmoregulation mechanism of *Ae. aegypti* has an average hemolymph osmolarity in the freshwater of about 250 osmoles l<sup>-1</sup> (Hiscock et al. 2002). The larvae's capacity to regulate the osmotic pressure of salinity shows a difference in the hemolymph reaction (Edwards 1982b). Larva *Ae. aegypti* coastal areas tolerated a maximum salinity of 18 ppt (500 mOsm), more than 18 ppt larvae occurred osmoconformers (Garrett and Bradley 1984). Larva *Ae. aegypti* with low concentrations of artificial seawater, the media osmotic pressure approached hemolif. Hemolific osmotic pressure increases sharply when the concentration of salinity water increases (Edwards 1982a; Richards and Meier 1974). Salinity-tolerant arboviral disease vectors are a health problem in humans in the world (Rezza et al. 2007; Pfeffer and Dobler 2010). Adaptation of vectors in freshwater to brackish water can increase disease transmission by mosquitoes in coastal areas (Ramasamy and Surendran 2011). Anthropogenic-induced adaptive changes in vector mosquitoes can lead to range expansion,

facilitating large areas' invasion (Ramasamy and Surendran 2016).

Oviposition study in the laboratory, adult *Ae. aegypti* in coastal adapted to a salinity of 15 ppt, unlike adult *Ae. aegypti* from inland put eggs only at a salinity of 10 ppt. An eggs trapped in the ovitrap decreases if a higher salinity level. In general, adult *Ae. aegypti* in coastal Randomayang and inland Martajaya prefer to put eggs at a salinity of 3 ppt. According to the previous studies, colony oviposition of *Ae. aegypti* put eggs at a salinity of 1, 2.5, and 18 ppt (Rammasamy et al. 2011; Panigrahi et al. 2014; Yap et al. 1995).

In this study, the results of Person analysis with a significance value of 0.01 showed that the survival rate of instar larva I, II, III, and IV in both habitat ecosystems significantly showed a relationship to the adaptation of each salinity level with  $p < 0.001$ . According to Rammasamy et al. (2011), the survival of larvae in inland and coastal ecosystems has a significant value for salinity with a p-value of 0.01. In the research of Mukhopadhyay et al. (2010), the survival rate of larvae with NaCl with a salinity concentration of 1.50% is directly related to larval mortality of 50%-90% at 19-29 hours. Salinity can affect the size of the anal papillae in larvae *Ae. aegypti* (Surendran et al. 2018). According to Kengne et al. (2019), the adaptability of *Ae. aegypti* larvae to salinity show a correlation in larval hemolymph in maintaining osmolarity with salinity tolerance with P-value  $< 0.05$ .

This study concluded that no current evidence for the large-scale adaptation of *Ae. aegypti* undergoing preimaginal development in natural brine. But on a laboratory scale, *Ae. aegypti* can survive, tolerate salinity, and put eggs of up to 15 ppt. Until recently, the application of vector control methods still prioritizes freshwater habitats in urban environments. Brackish water on abandoned in coastal Randomayang could change the adaptation pattern of *Ae. aegypti* in the future. This change is a serious concern regarding health since the high dengue hemorrhagic fever is associated with *Ae. aegypti* adapted in inland and coastal of Pasangkayu District. Data on the availability of *Ae. aegypti* habitats in this overlooked area should be warning the vector control program that coastal areas need to be a concern, especially dengue fever because this outbreak can occur at any time.

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