Effect of gradual salinity acclimatization on survival and physiological health of *Litopenaeus vannamei* post larvae in freshwater

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Abstract. This study aimed to evaluate the effect of gradual salinity acclimatization on the survival and physiological health of *Litopenaeus vannamei* post larvae (PL12) in freshwater culture. Acclimatization was conducted by gradually reducing salinity from 12 to 0.5 ppt through three treatments with durations of 5, 10, and 15 days. The results showed that the gradual decrease in salinity did not have a significant effect ($p > 0.05$) on survival and post larval growth between treatments, with the highest survival rate of 88.61% in the 5-day treatment (T1_5D). In contrast, the control treatment (CT_15D) with drastic salinity reduction in one stage showed significant differences ($p < 0.05$), with a survival rate of 24.0%. Physiological health parameters, including hepatopancreas condition, lipid levels, and gut-to-muscle ratio, remained within normal limits throughout the acclimatization process. The gradual decrease in salinity was also effective in maintaining the health of the vital organs of the post larvae and protecting them from pathogen infection. Overall, gradual acclimatization proved important in maintaining the survival and physiological health of *L. vannamei* during the transition to a freshwater environment.
Key Words: environment, gut to muscle ratio, hepatopancreas, reducing salinity.

Introduction. Pacific white shrimp, *Litopenaeus vannamei*, is one of the leading commodities in the fisheries sector due to its ability to adapt to various salinity levels, with a tolerance range of 0.5 to 45 ppt (Chong-Robles et al 2014). Its excellent osmoregulatory ability allows it to grow optimally in a wide range of conditions, making it one of the most widely farmed species globally. In 2020, the total aquaculture production of *L. vannamei* in the world was about 5.5 million tons, and China, Indonesia, Ecuador, Vietnam, and Thailand are known to be the major countries producing this species (FAO 2022).

L. vannamei farming in freshwater systems is increasingly in demand as an alternative to inland aquaculture, as this species has excellent adaptability to various salinity levels. According to research, if given high-quality feed and proper environmental management, *L. vannamei* can grow optimally in freshwater, equivalent to their growth in brackish water (Araneda et al 2008). Araneda et al (2008), Scabra et al (2023) and Supono et al (2023) have successfully cultivated *L. vannamei* in freshwater. Low-salinity aquaculture technologies can increase production efficiency while providing economic and ecological benefits. The use of freshwater or low-salinity water can reduce operational costs (Sakas 2016). Additionally, cultivating shrimp at low salinity reduces the risk of pathogens like *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV), commonly found in high salinity environments, proliferating. Results show that low salinity reduces the likelihood of WSSV outbreaks by reducing pathogen load in pond water and improving shrimp health conditions (Zhang et al 2016; Bauer et al 2021). This reduction in disease risk increases productivity and reduces the use of antibiotics and chemicals, thereby supporting environmental sustainability (Liu et al 2023).

However, even though *L. vannamei* can adapt to a wide range of salinity levels, proper acclimatization is critical in the production process, especially at low salinities. The acclimatization process from high to low salinity is complex and depends on the appropriate rate of salinity reduction to reduce osmotic stress and improve survival (Jayasankar et al 2009; Roy et al 2010). McGraw & Scarpa (2004) demonstrated that extending the acclimatization duration from 48 hours to 72 hours can improve the survival of freshwater-adapted shrimp. In addition, the health of the shrimp hepatopancreas, which plays an important role in digestion and metabolism, also affects the success of acclimatization. Damage to the hepatopancreas is often an early indication of infection with diseases like acute hepatopancreatic necrosis disease (caused by *V. parahaemolyticus*) and WSSV virus that can worsen the physiological condition of shrimp (Tran et al 2013).

In freshwater environments, absorption of minerals such as calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and sodium (Na^+) is a major challenge for shrimp, which can affect growth and molting. Research has shown that mineral supplementation in feed or culture water can improve shrimp's mineral uptake and survival in freshwater. Davis et al (2002) reported that post larvae reared in low salinity well water at 4 ppt, supplemented with KCl and $MgCl_2$, had a higher survival rate than animals reared in low salinity water without mineral supplements. Additionally, maintaining optimal water quality, which includes pH, temperature, dissolved oxygen, and ammonia concentration, is crucial for shrimp health, as ionic imbalances in freshwater can disrupt osmoregulation and increase the risk of mortality (Lin & Chen 2001; Cheng et al 2006).

This study's goal was to find out how gradual salinity acclimatization affected the health and survival of *L. vannamei* post larvae in freshwater culture. *L. vannamei* can live in a wide range of salinity levels because they are euryhaline. However, if the salinity drops too quickly, it can cause osmotic stress, which raises the risk of death due to osmoregulatory imbalances (Davis et al 2002). Previous studies have shown that gradual decreases in salinity can maintain the physiological health of shrimp, including the condition of the hepatopancreas which plays an important role in metabolism and defense against pathogens such as *Vibrio* spp. (Jayasankar et al 2009). Thus, this study will evaluate the optimal timing of salinity reduction, as well as its impact on the physiological health and survival of *L. vannamei* in a freshwater environment.

Material and Method

Aclimatization. The experiments were performed at the Indoor Culture Facility of PT Mochtar Sani Corporation (MSC), located in Bandar Lampung, Lampung, Indonesia. This study was conducted from December 2023 to January 2024. The experimental animals, *L. vannamei* post larvae (PL), were obtained from a commercial shrimp hatchery at Kalianda, East Lampung, Lampung. Nine-day-old post larvae (PL9) were received that had acclimated to 12 ppt salinity in the hatchery and three days of acclimation in a 2000 L rectangular concrete tank to produce PL12. The use of PL12 was based on the common practice of local shrimp farmers when stocking post larvae into ponds (Abrori et al 2022).

Experimental design. This study used a completely randomized design with three treatments and one control, each with three replications. The treatments involved variations in the duration of salinity reduction: T1 (5 days with a decrease of 2.4 ppt per day), T2 (10 days with a decrease of 1.2 ppt per day), and T3 (15 days with a decrease of 0.8 ppt per day), which gradually decreased the initial salinity from 12 ppt to 0.5 ppt. In the control treatment (CT), post larvae (PL) were maintained at 12 ppt, and salinity was drastically reduced to 0.5 ppt on day 15 (Figure 1). Salinity reduction was done by reducing the volume of water and adding fresh water according to the calculation. McGraw & Scarpa (2004) showed that shrimp survival can be increased by extending the duration of acclimatization, so the duration in this study was extended longer than 72 hours.

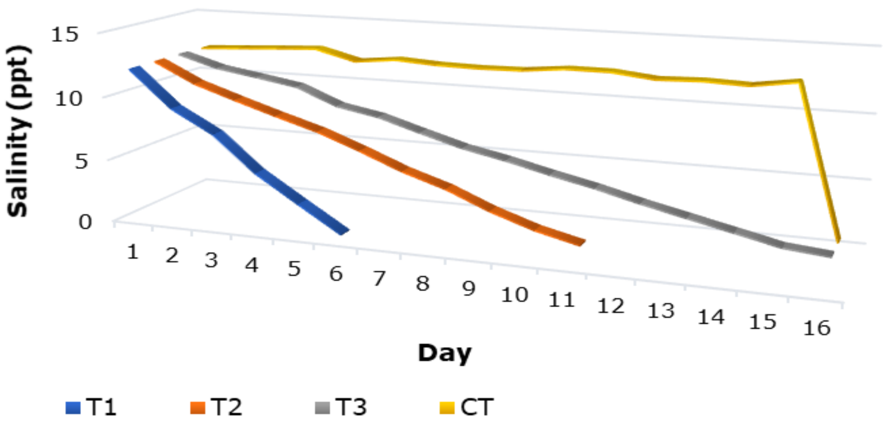


Figure 1. Graph of gradual reduction of salinity (treatments T1, T2 and T3) and drastic salinity reduction (control treatment CT).

Research procedures and data collection. Evaluation of growth and survival was conducted at the beginning and conclusion of the study by weighing a sample comprising 10% of each replicate and counting the surviving *L. vannamei*. Physiological health parameters of the experimental animals, including hepatopancreas condition, lipid levels, and GMR, were also evaluated. The percentage of tubules, hepatopancreas lipid content, and GMR were determined from 10% of the samples taken from each replicate. In addition, the total bacteria count (TBC) in the media water and the total *Vibrio* count (TVC) from the culture water and post larvae bodies were also calculated, which were analyzed for each replication. Water quality parameters, including dissolved oxygen (DO), temperature, pH, salinity, conductivity, and total dissolved solids (TDS), were measured daily in the morning using a water quality meter (Merck AZ Instrument type 86031), while alkalinity was analyzed following the APHA (2017) method. Ammonia and nitrite concentrations were measured using a spectrophotometer (Perkin Elmer UV/VIS Lambda 35) in accordance with procedures defined by SNI standards (SNI (2005) for ammonia; SNI (2004) for nitrite). Additionally, mineral content analysis, including Mg^{2+}, K^{+}, and Ca^{2+}, was performed to ensure the maintenance of an optimal culture environment. The concentrations of Mg^{2+}, K^{+}, and Ca^{2+} were determined using an atomic absorption spectrophotometer (AAS) according to the ASTM E663-86 standard and the Shimadzu AA-7000 AAS operation manual.

Statistical analysis*.* Research data including survival rate, weight gain, body health condition, TBC and TVC, mineral content, and water physicochemical parameters were analyzed using the ANOVA (Analysis of Variance) method, followed by the Tukey test at the 95% confidence level. Prior to ANOVA analysis, normality and homogeneity of variance tests were conducted first to ensure the validity of the analysis results. The tabulated data were then analyzed using MS Excel version 2019 and SPSS version 26.

Results

Survival rate of post larvae. Table 1 shows the survival rates and average daily growth (ADG) of *L. vannamei* after varying salinity acclimatization periods. The results indicate that a gradual reduction in salinity generally led to higher survival rates compared to a one-step acclimatization process. Statistical analysis revealed no significant differences ($p > 0.05$) in survival rates among the gradual acclimatization treatments (T1, T2, and T3). However, there was a significant difference ($p < 0.05$) in the survival rate between the stepwise acclimatization treatment and CT. A significant difference was also obtained in the average daily growth between treatment T1 and the control (CT) but not significant in treatments T2 and T3. Additionally, the table demonstrates a trend of increasing post larvae body weight in correlation with the 15-day rearing period.

Table 1

Survival and growth of *L. vannamei* during rearing

Parameters	*Treatments*			
	CT	*T1*	*T2*	*T3*
Survival rate (%)	24.0±6.0^{b}	88.61±4.59^{a}	80.3±1.73^{a}	79.4±4.28^{a}
ADG (g day^{-1})	1.27±0.04^{a}	0.42±0.12^{b}	0.89±0.10^{ab}	1.13±0.06^{ab}

Note: different superscripts in the same row indicate significant differences between treatments ($p < 0.05$); the values are means±standard deviation; ADG = average daily growth.

Physiological health parameters. Table 2 shows the health parameters of *L. vannamei* post larvae that were observed throughout the study. The results indicate that there were no significant differences in the percentage of hepatopancreatic tubules and lipid levels from the initial observation to the conclusion of the study across treatment groups. Similarly, the GMR of *L. vannamei* did not show any significant variations between treatments. These findings suggest that the applied treatments did not have a substantial impact on the physiological health parameters of *L. vannamei* post larvae over the course of the study.

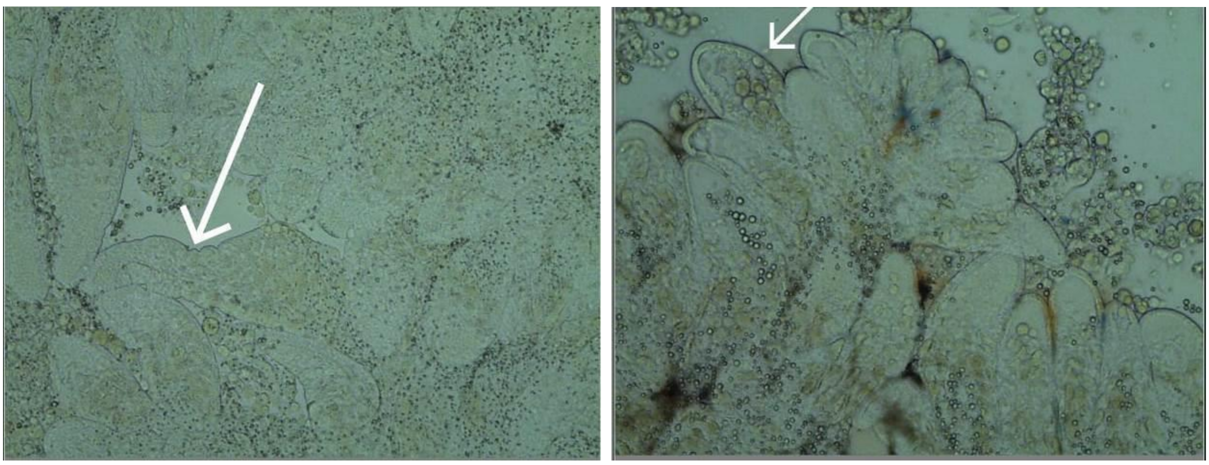
Table 2

Healthy parameters of body of *Litopenaus vannamei* culture

Parameters	*Initial*	*Treatments*			
		CT	*T1*	*T2*	*T3*
Hepatopancreatic tubules (%)	70.33±1.53	84.17±11.20^{a}	74.33±8.14^{a}	83.33±7.11^{a}	87.08±5.05^{a}
Lipid (%)	73.33±0.58	82.08±8.32^{a}	72.33±3.06^{a}	86.67±6.17^{a}	80.42±11.81^{a}
GMR	2.45±0.05	2.58±0.14^{a}	2.47±0.09^{a}	2.47±0.13^{a}	2.39±0.13^{a}

Note: the values are means±standard deviation.

In this study, some shrimp were observed to be in poor condition (Figure 2a), while the majority were in good health (Figure 2b). The shrunken hepatopancreas in Figure 2a (indicated by the arrow) suggests that these shrimps were experiencing abnormal physiological conditions or health issues related to this organ. Potential factors contributing to hepatopancreatic shrinkage include environmental stress, infection, or suboptimal water quality. In contrast, Figure 2b depicts shrimp with a normal, healthy hepatopancreas, exhibiting no signs of shrinkage or damage. This suggests that the shrimp thrived in an environment that supported their optimal physiological function.



a **b**

Figure 2. The difference in hepatopancreas tubules (indicated by arrows) in poor condition (a) and in good condition (b) of *L. vannamei*.

Figure 3 illustrates the ratio between muscle (indicated by the long blue arrow) and gut (indicated by the short black arrow) in post larvae of *L. vannamei*, which is approximately 3:1. This ratio reflects a healthy and balanced proportion between muscle mass and gut size, signifying adequate digestive capacity necessary for optimal growth. An ideal GMR further suggests that the post larvae are in excellent condition, receiving sufficient nutrient intake to support both robust muscle development and overall physiological health.

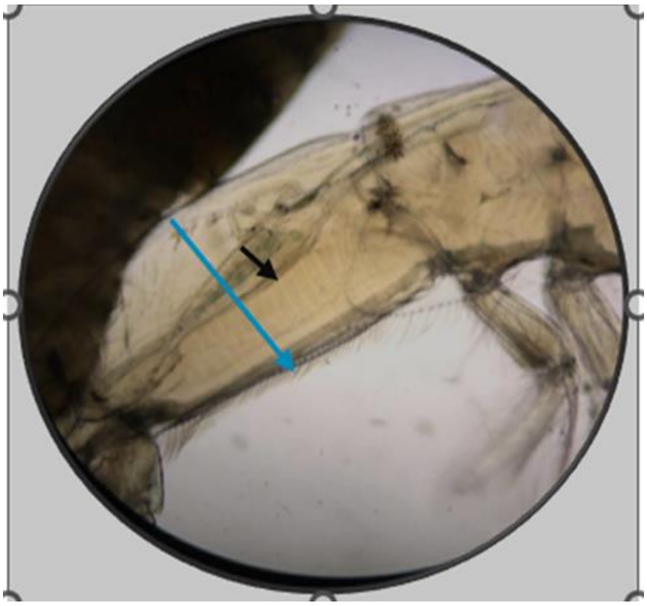


Figure 3. Comparison between muscle (long blue arrow) and gut (short black arrow) of PL 12 *L. vannamei*.

Table 3 presents the changes in TVC and TBC in the culture water and the bodies of post larval shrimp during the rearing period. In general, TVC and TBC in the culture water increased over time, with a notable rise observed on days 10 (T2) and 15 (T3). The TVC in the culture water for both treatments was higher compared to the control, with the highest count recorded in treatment T2 (607 CFU mL^{-1}). Conversely, in all treatments except the control, *Vibrio* spp. levels in the shrimp body were very low or undetectable, with the control treatment displaying the highest count (260 CFU mL^{-1}). The TBC in the water showed significant variation, with treatment T3 having the highest count (830 CFU mL^{-1}), while the control had the lowest count (330 CFU mL^{-1}). Furthermore, on days 10 and 15, the TVC/TBC ratio in the water increased, indicating a relative increase in *Vibrio* spp. prevalence compared to the total bacterial count in the culture environment.

Table 3

Total bacteria and *Vibrio* spp. count in the culture of *Litopenaeus vannamei*

Parameters	*Initial*	*Treatments*			
		CT	*T1*	*T2*	*T3*
TVC water (CFU mL^{-1})	26.0±0.0	7.0±1.0^{c}	0.0±0.0^{c}	607±83^{a}	226±16^{b}
TVC body (CFU mL^{-1})	260.0±0.0	9.3±15.31^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
TBC water (CFU mL^{-1})	600±0.0	330±29.5^{d}	170±41.6^{c}	695±15^{b}	830±10^{a}
Ratio TVC/TBC water	31.71±0.0	2.04±0.65^{c}	0.0±0.0^{c}	87.5±13.15^{a}	27.21±1.60^{b}

Notes: TVC = total *Vibrio* count; TBC = total bacteria count; different superscripts in the same row indicate significant differences between treatments (p < 0. 05); the values are means±standard deviation.

Table 4 presents the mineral parameters of the culture water throughout the study. The results indicate a significant decline in the concentrations of key minerals, including magnesium (Mg^{2+}), potassium (K^{+}), and calcium (Ca^{2+}). The initial magnesium concentration of 103.9 mg L^{-1} dropped sharply, with the lowest concentration recorded in treatment T3 at 6.08 mg L^{-1}. Similarly, the initial potassium concentration of 27.54 mg L^{-1} decreased to its lowest value of 8.073 mg L^{-1} in the CT. Calcium, which started at 76.88 mg L^{-1}, also showed a notable reduction, reaching a minimum value of 19.47 mg L^{-1} in the T3 treatment. The results show that the amount of minerals in the culture water dropped a lot during the acclimatisation period. This could be because the post-larvae absorbed minerals or because of other things that changed the minerals' availability in the water.

Table 4

Mineral parameters of water of *Litopenaus vannamei* culture

Parameters	*Initial*	*Treatments*			
		CT	*T1*	*T2*	*T3*
Mineral Mg (mg L^{-1})	103.9±16.58	56.463±0.29^{a}	14.85±4.53^{b}	14.86±0.96^{b}	6.08±3.16^{c}
Mineral K (mg L^{-1})	27.54±1.07	8.073±0.04^{b}	13.34±2.84^{a}	8.41±1.85^{b}	9.72±2.28^{b}
Mineral Ca (mg L^{-1})	76.88±0.00	24.083±0.74^{a}	29.47±4.40^{a}	21.21±1.65^{ab}	19.47±1.16^{b}

Note: different superscripts in the same row indicate significant differences between treatments ($p < 0.05$); the values are means±standard deviation.

An overview of the various water quality parameters during the culture period (Table 5), shows that DO and temperature were relatively stable in all treatments, while pH decreased slightly over time, but was still within the acceptable range for shrimp culture. Following the decrease, salinity remained relatively stable in all treatments, with the lowest value in treatment T3 (0.51 ppt). Conductivity and TDS also decreased between treatments. Water alkalinity increased in treatments T2 and T3 compared to the control, indicating changes in water quality during the acclimatisation process. Fluctuations in ammonia levels were observed, with the highest concentration recorded in the T3 treatment; however, this was not significantly different from the control (CT). In contrast, the control treatment (CT) had the highest nitrite levels, showing a significant difference from T1 but no significant difference with T2 and T3. These data indicate changes in the physicochemical parameters of the water during the acclimatisation process, which may affect the environmental conditions and health of *L. vannamei*.

Table 5

Healthy parameters of body of *Litopenaus vannamei* culture

Parameters	*Initial*	*Treatments*			
		CT	*T1*	*T2*	*T3*
DO (mg L^{-1})	4.64±0.17	4.64±0.17^{a}	4.81±0.1^{a}	4.71±0.11^{a}	4.58±0.06^{a}
Temperature (^{o}C)	30.58±0.16	30.33±0.60^{a}	30.34±0.75^{a}	30.45±0.25^{a}	30.59±0.26^{a}
pH	8.35±0.17	8.02±0.18^{b}	8.35±0.09^{a}	8.24±0.11^{ab}	8.13±0.07^{ab}
Salinity (ppt)	12.41±0.48	0.53±0.02^{a}	0.55±0.04^{a}	0.53±0.02^{a}	0.51±0.04^{a}
Conductivity (µS cm^{-1})	20.29±0.32	1078.9±80.19^{a}	1022.0±68.17^{a}	1006.3±21.73^{a}	937.0±60.83^{a}
TDS (mg L^{-1})	10.13±0.14	539.1±39.29^{a}	509.7±35.24^{a}	502.9±11.38^{a}	465.3±33.47^{a}
Alkalinity (mg L^{-1})	144±0.0	58.7±2.52^{b}	63.3±5.78^{ab}	76.8±8.31^{a}	70.8±9.06^{ab}
Ammonia (mg L^{-1})	0.25±0.0	0.24±0.02^{a}	0.25±0.0^{a}	0.5±0.0^{a}	1.83±1.26^{a}
Nitrite (mg L^{-1})	0.0±0.0	1.85±0.05^{a}	0.1±0.0^{b}	1.0±0.0^{ab}	1.67±1.15^{a}

Notes: different superscripts in the same row indicate significant differences between treatments ($p < 0.05$); the values are means±standard deviation.

Discussion

Survival rate and growth. The process of acclimatization of *L. vannamei* post larvae from a high-salinity environment to freshwater is a crucial challenge in aquaculture, given the shrimp's osmoregulatory ability that must be optimized. Osmoregulation is the ability of an organism to maintain the balance of ions and body fluids against its environment and in the face of drastic changes in salinity when transferred to a freshwater environment (Lignot et al 2000; Chong-Robles et al 2014). This study gradually carried out the acclimatization process, reducing the salinity from 12 ppt to 0.5 ppt over 5 days (T1), 10 days (T2), and 15 days (T3).

The percentage of survival of *L. vannamei* acclimatized with different times shows that gradual changes in salinity do not affect survival rates. This is because the shrimp have enough time to adapt to environments with varying salinities. Conversely, when acclimatisation was done drastically in one stage caused a decrease in survival rate. Statistical analysis showed that there was no significant difference ($p > 0.05$) in *L. vannamei* survival among treatments (T1, T2, and T3). However, there was a significant difference ($p < 0.05$) between treatments and control (CT). Meanwhile, body weight gain data showed that there was no significant difference between the CT and the T3 treatment. This finding is consistent with the research of Abrori et al (2022), which showed that gradual acclimatisation for 6 days can increase shrimp fry survival due to stable ion balance in the haemolymph, which helps reduce osmoregulatory stress (McGraw & Scarpa 2004). In contrast, in controls subjected to drastic salinity reduction, the mortality rate reached 76%. This supports the findings of Jayasankar et al (2009), that sudden changes in salinity can reduce shrimp survival to 20% within a few hours, and after 24 hours, none survived. An extreme ionic imbalance between the environment and hemolymph causes this condition, which the shrimp cannot cope with in a short time (Huong et al 2010).

The growth performance of *L. vannamei* also did not experience a significant decline during the gradual acclimatization process. During the maintenance period, the post larvae of *L. vannamei* increased in all treatments over 5, 10, and 15 days, starting from an average initial weight of 0.0056 g per individual, which then increased to an average weight of 0.0333 g per individual (T1), 0.1080 g per individual (T2), and 0.1932 g per individual (T3), as well as 0.2175 in the CT. This indicates that *L. vannamei* are still able to grow optimally despite variations in salinity. This finding is supported by the results of research by Ponce-Palafox et al (1997), which showed that salinity variations do not affect the growth of post-larvae shrimp within a period of 40 days. This finding reinforces the importance of handling acclimatization to ensure stable growth and optimal survival, especially in freshwater environments (Seethalakshmi et al 2021).

Healthy post larva. The study found that the acclimatization treatments did not make a big difference in the percentage of hepatopancreatic tubules, the levels of lipids, or the GMR. Stability in the gradual acclimatization process from salinity of 12 ppt to 0.5 ppt does not negatively impact the physiological health of *L. vannamei*. This result is in line with the findings of Wang et al (2023), that vital organs such as the hepatopancreas are able to adapt well during a gradual decrease in salinity, without causing excessive stress.

In shrimp, the hepatopancreas is the main organ in metabolism and digestion (Wang et al 2023). It showed stable values across all treatments during our study. Research by Wang et al (2023) supports these findings by stating that the gradual acclimatization method is able to reduce the stress of osmoregulation so that hepatopancreas function remains optimal in supporting metabolic and digestive processes during salinity reduction. This lets the hepatopancreas keep working at its best to support metabolic and digestive processes while the salinity level drops. In shrimp, the hepatopancreas plays a role in maintaining the efficiency of digestion and energy metabolism, especially under conditions of significant environmental change. In addition, the lipid levels, which reflect the shrimp's energy reserves and nutritional status, did not show significant changes indicating environmental alterations during the acclimatization process; however, the shrimp were able to maintain the energy reserves needed to support physiological functions. Huang et al (2019) reported that lipids, particularly triglycerides and phospholipids, play a role in the osmoregulation process of shrimp in coping with changes in salinity. This is because lipids provide the energy needed to maintain physiological functions and cell membrane structure during changes in environmental conditions.

The GMR, which is an indicator of digestive capacity and muscle growth, also remained stable in all treatments. These results indicate that the acclimatization process did not interfere with the digestive function and growth of the shrimp. This ratio is important to ensure that shrimp have the digestive capacity to support healthy and optimal growth. Peregrino (2006) stated that the proportion of muscle in the gut is another relevant parameter for assessing the quality of postlarvae. Maintaining this proportion in a 3:1 ratio serves as an indicator of adequate nutritional status. Overall, this study confirms that the stepwise acclimatization method is effective in maintaining the physiological stability of *L. vannamei* during the transition to a freshwater environment. This process allows shrimp to maintain the health of vital organs, such as the hepatopancreas, and the energy reserves necessary to support their adaptation without compromising growth and metabolic function. Research conducted by Lin & Chen (2001) also supports these findings, especially the osmoregulatory ability of *L. vannamei* in various salinity levels, which allows them to maintain metabolic balance and health during the acclimatization process.

Total Vibrio and bacterial counts. Based on the observations in Table 3, there has been a significant change in the total number of *Vibrio* and total bacteria (TVC and TBC) in the water and the post-larvae bodies of *L. vannamei* during the rearing period. Over time, the number of TVC and TBC in the water has increased, particularly on the 10th day (T2) and the 15th day (T3). The increase in the number of *Vibrio*, peaking at treatment T2 (607 CFU mL^{-1}), is higher compared to the control (7 CFU mL^{-1}). This increase is most likely due to the accumulation of organic matter from feed and metabolic waste, which provides a substrate for the growth of pathogenic bacteria such as *Vibrio* spp. (Seethalakshmi et al 2021). This condition is consistent with previous research, which suggests that an increase in organic matter in aquaculture systems can lead to the proliferation of pathogenic bacteria. On the contrary, the number of *Vibrio* in the shrimp's body is very low or even undetectable in all treatments except for the control, with the control recording the highest value of 260 CFU mL^{-1}. This indicates that the gradual acclimatization process (T1, T2, T3) helps protect shrimp from pathogenic bacterial colonization. Seethalakshmi et al (2021) reported that gradual acclimatization can enhance the immune response of shrimp, which ultimately helps prevent bacterial infections such as *Vibrio* spp. The gradual acclimatization process allows for osmoregulatory stability, which plays a crucial role in maintaining the health of shrimp during salinity changes, especially in low salinity environments.

The TBC at the highest water level occurred in treatment T3 (830 CFU mL^{-1}), reflecting the accumulation of microorganisms during the cultivation period. However, the *Vibrio* levels in the post larval shrimp body were lower in all treatments except for the control, indicating that, although there was an increase in total bacteria in the water, gradual acclimatization helped maintain the shrimp's health. This fits with what Seethalakshmi et al (2021) found. They say that keeping an eye on and controlling water quality factors like DO, pH, and salinity can help balance good microbes. This will stop the growth of pathogens like *Vibrio* spp., which are commonly the cause of shrimp diseases.

The increase in the ratio of TVC to TBC on the 10^{th} and 15^{th} days indicates the dominance of *Vibrio* in the cultural environment. However, the shrimp's health remains unaffected, as the gradual acclimatization process did not detect *Vibrio* in their bodies. The correct acclimatization process can help shrimp maintain their physiological balance and cope with microbiological stress from the cultivation environment (Lin & Chen 2001). A decrease in salinity can increase pathogens such as *Vibrio parahaemolyticus*, but acclimatization can reduce physiological stress and maintain gut microbiota balance, making shrimp more resistant to infections. This process also helps maintain the immunological activity of shrimp during environmental transitions (Chang et al 2024). Overall, this data shows that gradual acclimatization treatment is helpful for keeping the *L. vannamei* body stable during changes in salinity and is a key part of lowering the risk of pathogenic bacteria infections like *Vibrio* spp.

Mineral (Mg^{2+}, K^{+}, and Ca^{2+}). Based on the data presented in Table 4, there was a decrease in the concentration of minerals magnesium (Mg^{2+}), potassium (K^{+}), and calcium (Ca^{2+}) during the acclimatization process of *L. vannamei* from high salinity to freshwater. The big drop in these minerals' levels, especially in treatment T3 shows that the shrimp and its environment are interacting. This is because the shrimp use these minerals for many physiological functions, like keeping their osmotic balance and building their exoskeleton. Magnesium, which decreased from 103.9 to 6.08 mg L^{-1} , is one of the minerals involved in the formation of the exoskeleton and maintaining ion balance through the process of osmoregulation (Davis et al 2002). According to Cheng et al (2006), shrimp use minerals from the water to adapt to low salinity conditions. This is supported by the fact that shrimps absorb magnesium. These results stress the importance of mineral osmoregulation in low salinity conditions. Potassium levels dropped from 27.54 to 8.073 mg L^{-1} in treatment T3. Potassium helps maintain the balance of ions in shrimp cells, which supports the osmotic pressure inside the cells (Roy et al 2010). During the acclimatization process, shrimps rapidly absorb potassium to maintain their physiological stability, particularly in supporting muscle and nerve function, as indicated by the decrease in potassium concentration (Huong et al 2010). This decrease in potassium can disrupt the osmoregulation of shrimp and potentially affect their survival in low-salinity environments if not managed properly. Calcium, which decreased from 76.88 to 19.47 mg L^{-1}, is also a vital mineral in the molting process and the formation of the shrimp's exoskeleton. Shrimp need a lot of calcium to support their molting cycle, especially in low-salinity environments where calcium is scarce (Davis et al 2002). This is why the calcium level drops during the acclimatization process.

The microbiological dynamics within the cultivation system can also link to the decrease in minerals over time. Changes in mineral concentration in aquaculture water are crucial for maintaining the balance of the aquaculture ecosystem, as microorganisms use these minerals for their growth and biological activity (Zhang et al 2020). Therefore, not only does shrimp's absorption factor influence the availability of minerals in the water, but microbiological activity can also have an impact. Proper mineral management in water media, such as the supplementation of magnesium, potassium, and calcium during the acclimatization process, will ultimately enhance the survival and growth of shrimp in freshwater conditions. In addition to supporting osmoregulation, the addition of minerals can maintain metabolic balance, allowing shrimp to optimally adapt to low salinity environments (Wang et al 2023).

Water quality. According to the data from this study, various physicochemical parameters of the water underwent significant changes during the acclimatization process of *L. vannamei*. During the study, measurements of DO and temperature stayed within acceptable ranges for the post-larval *L. vannamei* to survive in all treatments, especially to help their metabolism and growth. The DO levels above 4 mg L^{-1} are quite good for the survival of shrimp, and the ideal temperature for the growth of *L. vannamei* is around 30°C. The right temperature supports efficient metabolic processes, while sufficient oxygen is necessary for aerobic activities that support vital functions, such as growth and molting (Venkateswarlu et al 2019).

The pH parameter in this study also experienced a decrease, but it remained within the optimal range for *L. vannamei* farming, which is between 7.5 and 8.5 (Venkateswarlu et al 2019). The decomposition of organic matter and the respiratory activity of microorganisms in the cultivation medium can cause this pH decrease, but it does not significantly affect the shrimp's health. Maintaining pH stability within that range facilitates the optimal growth and development of shrimp.

The implementation of a gradual acclimatization process resulted in a stable decrease in salinity across all treatments, with the lowest value at T3 (0.51 ppt). The stability of salinity is very important to avoid osmotic stress, which can affect shrimp's health and survival during the transition from a high-salinity environment to a low-salinity one. The gradual acclimatization process allows shrimp post larvae to effectively adapt to changes in salinity without experiencing significant osmoregulatory stress (Chen et al 2015).

As salinity decreased, conductivity and TDS decreased across treatments, reflecting a reduction in the concentration of ions and dissolved particles in water. The conductivity in the control treatment reached 1078.9 μS cm^{-1}, which is higher compared to T1 (1022.0 μS cm^{-1}), T2 (1006.3 μS cm^{-1}), and T3 (937.0 μS cm^{-1}). This shows that a steady amount of dissolved ions is important for the osmoregulation process in *L. vannamei*, especially when things in the environment change, like when the salinity changes (Duan et al 2022). This decline indicates that shrimp are capable of adapting to lower salinity environmental conditions without experiencing metabolic disturbances caused by drastic changes in dissolved substance content.

This study's alkalinity tends to be stable and does not show fluctuations during the acclimatization process. Stable alkalinity helps maintain the acid-base balance required for osmoregulation and nitrogen excretion processes, which are critical for shrimp survival in aquaculture environments (Zhang et al 2023).

The fluctuations in ammonia and nitrite levels during the study reflect an increase in biological activity in the water, with the highest ammonia levels recorded in T3 (1.83 mg L^{-1}). The accumulation of organic matter and nitrogen excretion from the shrimp are responsible for this increase in ammonia. High ammonia levels pose a serious threat to shrimp health, especially at dangerous concentrations. According to Nan et al (2024), acute exposure to ammonia in *L. vannamei* can cause damage to the gill structure, such as filaments and blood vessels that function to transport oxygen. This damage triggers metabolic disturbances, oxidative stress, and apoptosis (programmed cell death), which can ultimately lead to the death of shrimp. Therefore, the ammonia levels remain within safe limits to prevent toxic effects that could be fatal for shrimp.

Conclusions. Gradual salinity acclimatization from 12 ppt to 0.5 ppt in *Litopenaeus vannamei* postlarvae did not have a significant effect on survival and growth ($p > 0.05$), with the highest survival rate of 88.61% achieved in the 5-day treatment (T1). In contrast, in the control treatment with drastic acclimatization after 15 days, there was a significant difference ($p < 0.05$) with a low survival rate of 24.0%.

Acclimatization treatments did not significantly impact the percentage of hepatopancreatic tubules, lipid levels, or gut-to-muscle ratios of *L. vannamei*. The gradual acclimatization process from 12 ppt to 0.5 ppt did not negatively impact the shrimp's physiological health.

The post-larvae of *L. vannamei* showed low or undetectable levels of *Vibrio* spp. in all treatments, except the control, indicating that freshwater culture effectively protects shrimp from bacterial colonization. This process also supports stable osmoregulation, maintains shrimp health during salinity changes, and helps reduce microbiological stress in the culture environment.

During acclimatization, the levels of essential minerals such as magnesium (Mg^{2+}), calcium (Ca^{2+}), and potassium (K^{+}) in freshwater *L. vannamei* rearing media water decreased. Mineral supplementation is important to maintain nutritional balance and support the optimal health and growth of *L. vannamei* in freshwater aquaculture.

Physico-chemical parameters, such as dissolved oxygen, temperature, pH, conductivity, and total dissolved solids, supported the adaptation of *L. vannamei* in freshwater. Alkalinity remained stable, supporting osmoregulation, but higher ammonia levels obtained in T3 treatment may pose a risk to shrimp health.

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Conflict of interest. The authors declare that there is no conflict of interest.

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