EFFECTS OF LOW SALINITY ON IMMUNE RESPONSES AND SURVIVAL OF JUVENILE *Litopenaeus vannamei*

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ABSTRACT

Salinity is a key environmental factor affecting physiological performance and immune responses in shrimp aquaculture, particularly during adaptation to low-salinity culture systems. However, information on how reduced salinity influences both cellular and humoral immune responses in juvenile *Litopenaeus vannamei* remains limited, making it necessary to evaluate immune-related responses under different salinity conditions. This study evaluated the effects of different salinity levels on survival, hemocyte profiles, and selected cellular and humoral immune responses of juvenile *L. vannamei*. Shrimp (2.50 ± 0.28 g) obtained from a commercial hatchery were reared at salinities of 3.5, 7, 14, and 28 g L^{-1} in triplicate for 14 days. Measured parameters included survival rate, total hemocyte count (THC), differential hemocyte count (DHC), phagocytic activity, phenoloxidase activity, and lysozyme activity. Salinity significantly affected survival and most immune-related parameters. Survival increased progressively with increasing salinity, reaching 86.67 ± 3.85% at 28 g L^{-1} and declining to 48.89 ± 5.88% at 3.5 g L^{-1}. THC was highest at 14 g L^{-1} (2.61 ± 0.12 × 10^4 cells mm^{-3}) and decreased markedly under reduced salinity conditions. Similarly, phagocytic and phenoloxidase activities were significantly reduced at lower salinities, whereas lysozyme activity was not significantly affected. Overall, suboptimal salinity conditions below 7 g L^{-1} imposed physiological stress on *L. vannamei* juveniles, leading to impaired immune responses and reduced survival.

KEYWORDS: hemocyte; immune response; salinity; shrimp; survival

ABSTRAK: Pengaruh Salinitas Rendah terhadap Respons Imun dan Kelangsungan Hidup Juvenil Udang Vaname (*Litopenaeus vannamei****)***

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Salinitas merupakan faktor lingkungan penting yang memengaruhi performa fisiologis dan respons imun dalam budidaya udang, khususnya selama adaptasi pada sistem budidaya bersalinitas rendah. Namun, informasi mengenai bagaimana penurunan salinitas memengaruhi respons imun seluler dan humoral pada juvenil ***Litopenaeus vannamei*** *masih terbatas, sehingga diperlukan evaluasi terhadap respons imun pada kondisi salinitas yang berbeda. Penelitian ini mengevaluasi pengaruh berbagai tingkat salinitas terhadap kelangsungan hidup, profil hemosit, serta respons imun seluler dan humoral terpilih pada juvenil* ***L. vannamei****. Udang dengan bobot 2,50 ± 0,28 g yang diperoleh dari hatchery komersial dipelihara pada salinitas 3,5; 7; 14; dan 28 g L^{-1} dengan tiga ulangan selama 14 hari. Parameter yang diamati meliputi tingkat kelangsungan hidup, total hemocyte count (THC), differential hemocyte count (DHC), aktivitas fagositik, aktivitas fenoloksidase, dan aktivitas lisozim. Salinitas berpengaruh nyata terhadap kelangsungan hidup dan sebagian besar parameter imun. Kelangsungan hidup meningkat seiring meningkatnya salinitas, dengan nilai tertinggi pada 28 g L^{-1} sebesar 86,67 ± 3,85% dan terendah pada 3,5 g L^{-1} sebesar 48,89 ± 5,88%. Nilai THC tertinggi diperoleh pada salinitas 14 g L^{-1} sebesar 2,61 ± 0,12 × 10^4 sel mm^{-3} dan menurun pada salinitas rendah. Aktivitas fagositik dan fenoloksidase juga mengalami penurunan nyata pada salinitas rendah, sedangkan aktivitas lisozim tidak menunjukkan perbedaan nyata. Secara keseluruhan, kondisi salinitas suboptimal di bawah 7 g L^{-1} menyebabkan* stres fisiologis, menekan respons imun, dan menurunkan kelangsungan hidup udang uji.

KATA KUNCI: hemosit; kelangsungan hidup; respons imun; salinitas; udang

INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, is one of the most important aquaculture commodities due to its high economic value, rapid growth, adaptability to intensive culture systems, and tolerance to a wide range of salinity conditions (Chang *et al*., 2025). Global production of *L. vannamei* has increased substantially in recent years, including in intensive brackishwater ponds and inland low-salinity aquaculture systems (FAO, 2024). The expansion of low-salinity shrimp farming has attracted considerable attention because it enables shrimp cultivation in inland areas with limited access to seawater and provides opportunities for integration with freshwater aquaculture species (James *et al*., 2024).

Salinity is a major environmental factor influencing shrimp physiology, osmoregulation, growth, and survival. The optimal salinity range for *L. vannamei* culture is generally between 22–26 g L^{-1} (Hassan *et al*., 2021; Okasha *et al*., 2025). However, reduced-salinity culture systems are increasingly practiced in many regions. Although *L. vannamei* is considered a euryhaline species, abrupt or prolonged exposure to suboptimal salinity may induce osmotic stress, disrupt ion balance, and impair physiological performance (Deris *et al*., 2022). In addition to physiological disturbances, hyposaline culture conditions may also increase disease susceptibility. Chuchird *et al*. (2024) reported a mass mortality event in low-salinity shrimp culture systems in Thailand associated with infection by *Photobacterium damselae* subsp. *damselae*, indicating that reduced salinity may compromise shrimp health and increase vulnerability to opportunistic pathogens.

Previous studies have shown that low salinity may reduce survival and immune competence in shrimp (Lin *et al*., 2025), while hemocytes play essential roles in shrimp immunity through mechanisms such as phagocytosis, encapsulation, melanization,

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and activation of the prophenoloxidase system (Chang *et al*., 2024). Alterations in hemocyte-related parameters may therefore reflect physiological stress and immune suppression under unfavorable environmental conditions. Although hematological and immune responses under low-salinity conditions have been widely investigated, many previous studies have focused on acute osmotic shock, moderate salinity reduction, or specific developmental stages. Information regarding the integrated responses of cellular and humoral immune parameters in juvenile *L. vannamei* during prolonged low-salinity adaptation remains limited. This stage is particularly important because the white shrimp juveniles showed progressive patterns in osmoregulatory development (Abrori *et al*., 2022). Furthermore, the salinity level at which reduced salinity begins to compromise both survival and immune function, particularly under very low-salinity conditions below 7 g L^{-1}, remains insufficiently defined.

Therefore, we hypothesized that prolonged exposure to extreme low-salinity conditions, particularly below 7 g L^{-1}, would exceed the osmoregulatory capacity of *L. vannamei* juveniles, forcing a physiological trade-off that significantly suppresses their integrated immune parameters and subsequently reduces survival. To test this hypothesis and address this research gap, the present study evaluated the effects of a 14-day low-salinity exposure gradient, ranging from 3.5 to 28 g L^{-1}, on survival, hemocyte profiles, and selected cellular and humoral immune responses of juvenile *L. vannamei*. By combining cellular indicators, including total hemocyte count (THC), differential hemocyte count (DHC), and phagocytic activity, with humoral enzymatic responses, including phenoloxidase activity, and lysozyme activities, this study provides an integrated assessment of salinity-induced immune impairment and identifies a potential practical threshold for juvenile shrimp culture under low-salinity conditions.

MATERIALS AND METHODS

Time and Location

The present study was conducted from September to December 2025. Shrimp rearing and the *in vivo* experimental trials were carried out at the Fish Health Laboratory, Department of Aquaculture, IPB University. Subsequent analyses were performed at specialized facilities within the same department; water quality parameters were evaluated at the Aquaculture Environment Laboratory, and humoral enzymatic responses were quantified at the Genetics and Reproduction Laboratory.

Experimental Design

A completely randomized design (CRD) was employed with four salinity treatments (3.5, 7, 14, and 28 g L^{-1}), each with three replicates. The 14-day experimental period was selected to assess hematological and immune alterations induced by salinity stress during the adaptation phase of shrimp under low-salinity culture conditions.

Animals and Rearing Conditions

8-day-old shrimp post-larvae (PL8) of *L. vannamei* were obtained from a commercial hatchery, PT Suri Tani Pemuka, Indramayu, West Java. Prior to the experiment, the shrimp were acclimated and reared under controlled conditions until they reached the desired experimental size. During the 35-day rearing period, shrimp were maintained in fiberglass tanks (200 × 100 × 40 cm) with a water depth of 20 cm at a stocking density of 1,000 individuals per tank. Shrimp were fed a crumble-type commercial diet Feng-Li 0 sourced from PT. Matahari Sakti, containing 35% crude protein, 6% crude fat, 4% crude fibre, 12% ash, and 12% moisture four times daily.

Once the shrimp reached the experimental size (2.50 ± 0.28 g), they were randomly transferred into glass aquaria (55 × 50 × 30

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cm) at a stocking density of 15 individuals per tank. Prior to treatment exposure, all shrimp were maintained at 28 g L^{-1} salinity; thereafter, salinity was gradually reduced by 50% at 24 h intervals until the target salinity levels (3.5, 7, and 14 g L^{-1}) were achieved.

During the 14-day experimental period, shrimp were fed a pellet-type commercial diet Feng-Li 1 from PT. Matahari Sakti, containing 35% crude protein, 6% crude fat, 4% crude fibre, 12% ash, and 12% moisture to apparent satiation four times daily at 07:00, 10:00, 14:00, and 18:00. Water quality was maintained through daily siphoning of waste and partial water exchange (40%) every three days. Continuous aeration was provided throughout the experiment to maintain adequate dissolved oxygen levels.

Water temperature and salinity were monitored daily, whereas dissolved oxygen, pH, and total ammonia nitrogen (TAN) were measured at the end of the experimental period. Dissolved oxygen was measured using a portable DO meter (DO-5510, Lutron Electronic Enterprise Co., Ltd., Taiwan). Meanwhile, total ammonia nitrogen (TAN) was analyzed using the phenate spectrophotometric method following APHA standard procedures (American Public Health Association [APHA], 2017) with a UV–Vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA).

Hemolymph Collection

At the end of the experiment, three shrimp were randomly sampled from each replicate aquarium for hemolymph collection. Approximately 0.1 mL of hemolymph was collected from the ventral sinus at the base of the first abdominal segment using a sterile 1 mL syringe preloaded with 0.3 mL of chilled anticoagulant solution prepared for crustacean hemolymph collection. Due to the limited hemolymph volume obtainable from individual juvenile shrimp (3,20 ± 0,5 g) and the need to perform multiple immune-related assays, the hemolymph collected from the three shrimp within the same replicate aquarium was pooled to obtain one composite sample. Therefore, each composite sample represented one replicate aquarium, resulting in three composite samples per salinity treatment. The samples were immediately transferred into sterile 2.0 mL microcentrifuge tubes, kept on ice, and subsequently used for total hemocyte count (THC), differential hemocyte count (DHC), phagocytic activity, phenoloxidase activity, and lysozyme activity analyses. Statistical analyses were performed using the replicate aquarium as the experimental unit (n = 3 per treatment).

Measured Parameters

Survival Rate

Survival rate was calculated using Equation 1:

$$SR = \left(\frac{N_t}{N_0}\right) \times 100 \quad \text{.....(1)}$$

Description:
SR = Survival rate (%)
N_t = Number of shrimps at the end of the the experiment (individuals)
N_0 = Number of shrimps at the beginning of the experiment (individuals)

Total Hemocyte Count (THC)

Hemolymph was homogenized, loaded onto a hemocytometer, and observed under a light microscope at 400× magnification. Total hemocyte count (THC) was determined using the appropriate dilution factor and expressed as cells mm^{-3}.

Differential Hemocyte Count (DHC)

Hemolymph smears were prepared on glass slides, air-dried, fixed with methanol (5 min), stained with Giemsa (± 15 min), and examined under a microscope (1000×). Hemocytes were classified into hyalinocytes, semi-granulocyte, and granulocyte, and were expressed as a percentage.

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Phagocytic Activity

A drop of hemolymph was mixed with a *Staphylococcus aureus* suspension and incubated for ± 20 min. Smears were prepared, fixed, and stained with Giemsa, then observed under a microscope (400×). Phagocytic activity was expressed as the percentage of active phagocytic cells against *S. aureus* cells.

Phenoloxidase Activity

Phenoloxidase (PO) activity was quantified based on the formation of dopachrome from L-DOPA oxidation, measured as optical density at 490 nm. Hemolymph samples were centrifuged at 3500 rpm for ± 10 min at room temperature, after which the supernatant was discarded, and the pellet was washed with 1 mL cacodylate–citrate buffer followed by re-centrifugation. The pellet was subsequently resuspended in 200 μL cacodylate–citrate buffer, and a 100 μL aliquot was incubated with 50 μL trypsin at 25–26 °C for ± 20 min. Thereafter, 50 μL L-DOPA (L-3,4-dihydroxyphenylalanine) was added and incubated for ± 5 min, followed by the addition of 0.8 mL cacodylate buffer. The control consisted of 0.1 mL of cell suspension, 0.05 mL of cacodylate buffer, and 0.05 mL of L-DOPA. Optical density was measured at 490 nm using a spectrophotometer (RT-2100C, Rayto Life and Analytical Sciences Co., Ltd., China).

Lysozyme Activity

Lysozyme activity was determined spectrophotometrically by measuring turbidity reduction using *Micrococcus lysodeikticus* as the substrate. Hemolymph (0.1 mL) was mixed with a bacterial suspension prepared in phosphate-buffered saline (0,4 mg/6,2 mL PBS), then transferred into a microplate. The decrease in absorbance was measured at 450 nm using a spectrophotometer (RT-2100C, Rayto Life and Analytical Sciences Co., Ltd., China).

Statistical Analysis

Data were tested for normality and homogeneity of variance prior to analysis. One-way analysis of variance (ANOVA) was performed to evaluate the effects of salinity, followed by Duncan's multiple range test for post hoc comparisons at a significance level of $p < 0.05$. All analyses were conducted using SPSS 16.0 statistical software. Results are presented as mean ± standard deviation.

Ethical Statement

Ethical approval was not required for this study because the experimental organism was an invertebrate species. Nevertheless, all experimental procedures were conducted in accordance with standard aquaculture animal-handling practices and the Indonesian regulation for culture of *L. vannamei* Permen KP No. 75 (Kementerian Kelautan dan Perikanan [KKP], 2016) to minimize handling stress during rearing and sampling.

RESULTS AND DISCUSSION

Salinity treatment significantly affected survival and several hematological and immune parameters of *L. vannamei* juveniles ($p < 0.05$). In general, reduced salinity conditions were associated with decreased survival, lower total hemocyte count, reduced phagocytic activity, and decreased phenoloxidase activity, whereas lysozyme activity remained relatively stable among treatments. The expansion of shrimp farming into inland areas with limited access to saline water has become increasingly widespread in many Asian countries, primarily due to the remarkable adaptability of *L. vannamei* to low-salinity culture conditions (Ismail *et al*., 2021). While low-salinity culture systems offer several advantages for shrimp aquaculture, including reduced dependence on coastal areas and the potential for integration with freshwater species (James *et al*., 2024), the present study demonstrated that reduced salinity conditions may adversely affect the

physiological and immune performance of *L. vannamei* juveniles. The consistent reduction in survival rate, total hemocyte count, phagocytic activity, and phenoloxidase activity observed at salinity levels below 7 g L^{-1} suggests that prolonged exposure to hyposaline conditions imposes substantial osmotic stress, increases metabolic demand, and compromises immune function in shrimp.

Survival Rate

Salinity significantly affected the survival rate of *L. vannamei* juveniles ($p < 0.05$), with survival increasing progressively with salinity. The highest survival rate was observed at 28 g L^{-1} (86.67 ± 3.85%), whereas the lowest survival occurred at 3.5 g L^{-1} (48.89 ± 5.88%) (Figure 1). The present results demonstrate that hyposaline exposure adversely affected shrimp survival and physiological adaptation.

The decline in survival rate under reduced salinity suggests that shrimp experienced osmotic stress, which required substantial energy expenditure for osmoregulation. Under suboptimal salinity, shrimp must continuously regulate ionic and osmotic balance between body fluids and the surrounding environment, thereby increasing metabolic demand and reducing the energy available for growth and physiological maintenance (Truong *et al*., 2023). Prolonged osmotic stress may impair physiological stability and increase the risk of mortality. Similar trends were reported by Gao *et al*. (2016), who observed that decreasing salinity levels were associated with lower survival of *L. vannamei* juveniles. Comparable findings were also reported by Xie *et al*. (2018), where an acute salinity reduction from 28 to 5 g L^{-1} significantly decreased the survival of *L. vannamei* post-larvae. The reduced survival observed in low-salinity environments may therefore reflect impaired osmoregulatory performance and physiological stress resulting from abrupt or prolonged exposure to hyposaline conditions. In addition, reduced salinity may indirectly compromise shrimp resistance to opportunistic pathogens by suppressing immune performance, as indicated by the reduction in hemocyte-related immune parameters observed in the present study.

Total Hemocyte Count (THC)

Salinity significantly influenced the total hemocyte count (THC) of *L. vannamei* juveniles ($p < 0.05$). The highest THC was recorded at 14 g L^{-1} (2.61 ± 0.12 × 10^4 cells mm^{-3}),

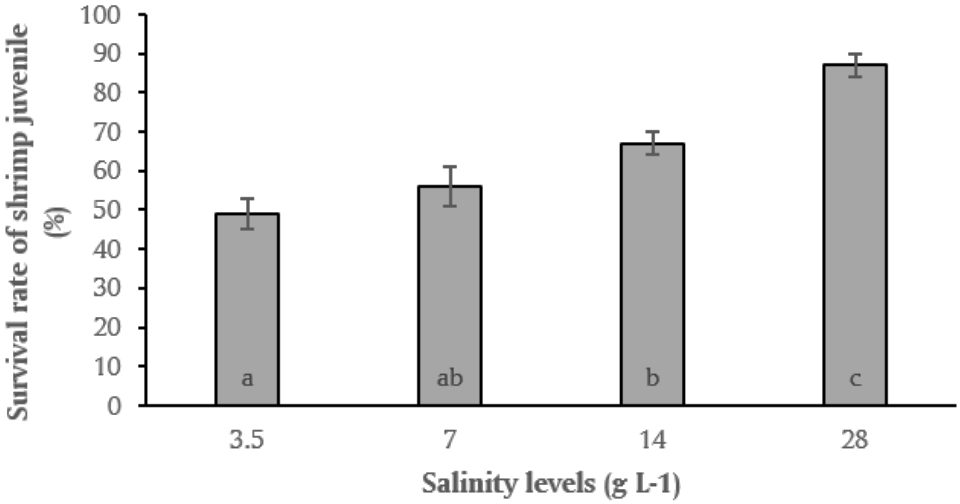


Figure 1. Survival rate of *L. vannamei* juveniles under different salinity levels for 14 days of observation (mean ± SD, n = 3). Different letters indicate significant differences among treatments based on Duncan's multiple range test ($p < 0.05$)

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whereas the lowest value was observed at 3.5 g L^{-1} (1.13 ± 0.05 × 10^4 cells mm^{-3}) (Figure 2).

Hemocytes are the primary cellular components involved in the innate immune system of crustaceans and play essential roles in pathogen recognition, phagocytosis, encapsulation, and activation of the prophenoloxidase system. According to Dewi *et al*. (2021), increased THC is associated with enhanced mobilization of hemocytes, contributing to improved non-specific immune responses in white shrimp after receiving Guava leaf extract (GLE) orally. Higher hemocyte abundance elicited by probiotics may increase the ability of shrimp to recognize and eliminate invading foreign particles or pathogens (Utami *et al*., 2025). Interestingly, THC at 28 g L^{-1} was slightly lower than that observed at 14 g L^{-1}, suggesting that immune responses may not increase linearly with salinity. This finding may indicate the existence of an optimal physiological range for hemocyte regulation rather than a simple positive relationship between salinity and immune performance. Similar variations in hemocyte responses across different salinity conditions have also been reported in previous studies on *L. vannamei* cultured under osmotic stress conditions (Abrori *et al*., 2022; Covarrubias *et al*., 2024).

Differential Hemocyte Count (DHC)

The differential hemocyte count (DHC) of *L. vannamei* juveniles consisted of hyalinocyte, semi-granulocyte, and granulocyte. Salinity treatment did not significantly affect the proportion of hyalinocytes and semi-granulocyte ($p > 0.05$). In contrast, granulocyte showed significant differences among treatments ($p < 0.05$), with the highest proportion observed at 3.5 g L^{-1} (33.33 ± 1.76%) and the lowest at 28 g L^{-1} (24.00 ± 2.31%) (Figure 3).

While total hemocyte count decreased under reduced salinity conditions, the relative increase in granulocyte suggests that shrimp may preferentially mobilize immune-related hemocyte types in response to osmotic stress, where alterations in hemocyte composition reflect physiological adaptation and immune modulation. In addition, Ye *et al*. (2024) reported that normal *L. vannamei* strains exhibited lower antioxidant levels and immune capacity under low-salinity conditions compared with hybrid strains, indicating that genetic background may influence salinity tolerance and immune resilience in shrimp cultured under hyposaline environments. In this study, the absence of significant differences in hyalinocyte and semi-granulocyte among treatments suggests that

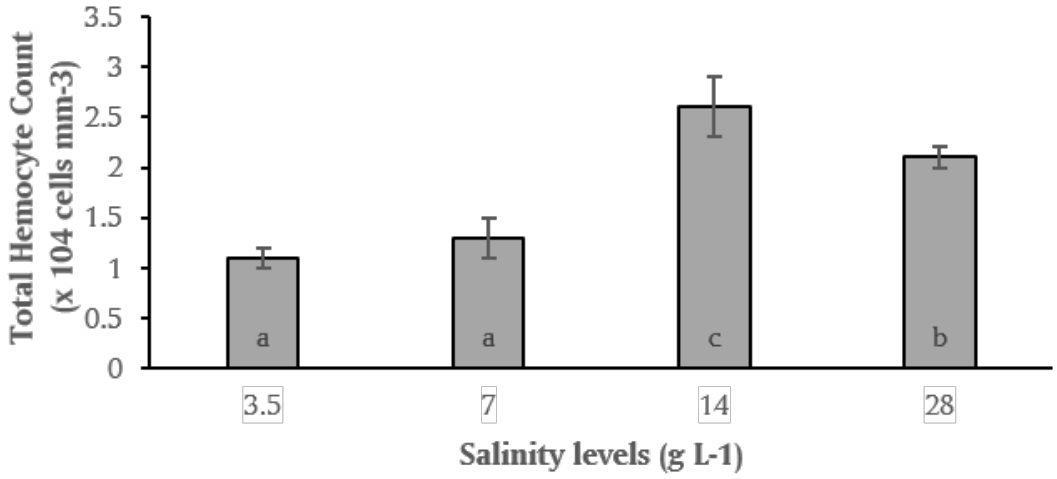


Figure 2. Total hemocyte count (THC) of *L. vannamei* juveniles under different salinity levels over 14 days of observation (mean ± SD, n = 3). Different letters indicate significant differences among treatments based on Duncan's multiple range test ($p < 0.05$)

these hemocyte types may be less sensitive to salinity variation or remain relatively stable during short-term osmotic stress exposure. The increase in granulocytes under low salinity may reflect compensatory immune mobilization in response to osmotic stress, since granular hemocytes are strongly associated with prophenoloxidase activation and defense reactions (Dewi *et al*., 2025). Overall, the observed changes in DHC indicate that salinity variation may alter hemocyte composition and immune regulation in *L. vannamei* juveniles.

Phagocytic Activity

Phagocytic activity of *L. vannamei* juveniles was significantly affected by salinity treatment ($p < 0.05$). The highest phagocytic activity was observed at 28 g L^{-1} (70.67 ± 5.70%), whereas the lowest value was recorded at 3.5 g L^{-1} (42.00 ± 2.31%) (Figure 4).

The reduced phagocytic activity observed in the present study was consistent with the decrease in total hemocyte count under low salinity, indicating that salinity stress

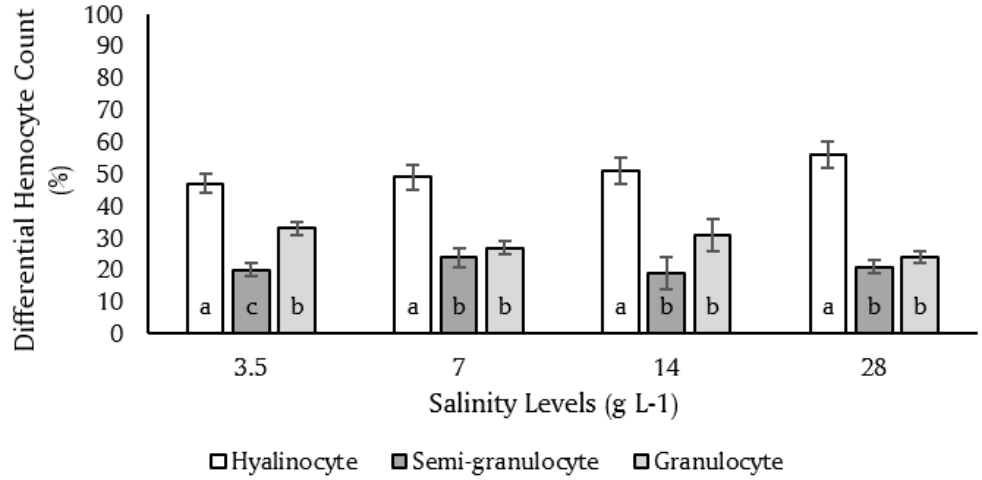


Figure 3. Differential hemocyte count (DHC) of *L. vannamei* juveniles under different salinity levels for 14 days of observation (mean ± SD, n = 3). Different letters indicate significant differences among treatments based on Duncan's multiple range test ($p < 0.05$)

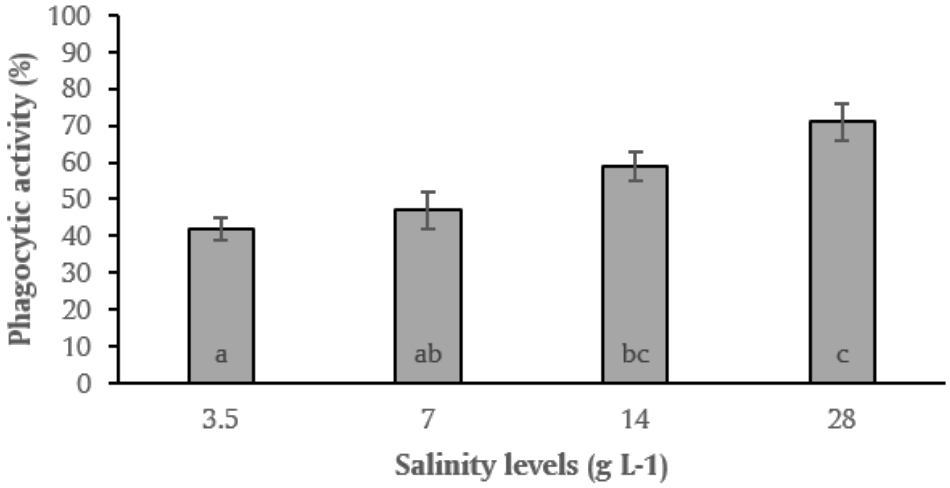


Figure 4. Phagocytic activity of *L. vannamei* juveniles under different salinity levels over 14 days of observation (mean ± SD, n = 3). Different letters indicate significant differences among treatments ($p < 0.05$)

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may simultaneously affect both hemocyte abundance and functional immune capacity. Lower hemocyte efficiency under osmotic stress conditions may consequently weaken the shrimp's non-specific immune defense and increase susceptibility to opportunistic infections. Similar findings have been reported in previous studies, where abrupt or prolonged salinity changes negatively affected immune responses and physiological stability in *L. vannamei* (Umiliana *et al*., 2016).

Phenoloxidase (PO) Activity

Phenoloxidase (PO) activity of *L. vannamei* juveniles was significantly affected by salinity treatment ($p < 0.05$). The highest PO activity was observed at 28 g L^{-1} (0.471 ± 0.026), whereas the lowest value was recorded at 3.5 g L^{-1} (0.129 ± 0.009) (Figure 5).

In the present study, the decline in PO activity was consistent with reductions in total hemocyte count and phagocytic activity under low salinity, indicating that salinity stress may broadly suppress cellular and humoral immune responses in *L. vannamei*. These findings further support the hypothesis that prolonged exposure to reduced salinity may compromise shrimp immune competence and increase susceptibility to disease. Osmoregulation is the main physiological mechanism that maintains homeostasis in crustaceans (Thabet *et al*., 2017). The increased energetic demand associated with these processes may consequently compromise immune performance, including reductions in hemocyte abundance and immune-related activities such as proPO cascades (Cheng *et al*., 2025).

Lysozyme Activity

Lysozyme activity of *L. vannamei* juveniles was not significantly affected by salinity treatment ($p > 0.05$), with activity values ranging from 0.409 to 0.445 (Figure 6). Although slight variations were observed among treatments, the results indicate that lysozyme activity remained relatively stable under different salinity conditions.

Lysozyme is an important humoral enzymatic component of the crustacean innate immune system (Hu *et al*., 2022). In the present study, however, lysozyme activity did not differ significantly between the low-salinity treatments and the control group ($p > 0.05$). This result suggests that lysozyme activity in juvenile *L. vannamei* remains relatively constant across the tested salinity range, indicating that its activation is tightly coupled with pathogen-induced stimulation rather than environmental fluctuations. Supporting this view, Subash *et al*. (2022) reported elevated lysozyme activity during the early phases of EHP infection in *L. vannamei*, and structural components like lipopolysaccharides (LPS) have been shown to modulate its activity in the hepatopancreas even without live pathogen exposure (Xu *et al*., 2026). At the molecular level, lysozyme gene expression also rises sharply following challenges with *Vibrio parahaemolyticus* (Hu *et al*., 2022).

In the absence of a pathogen challenge, the prolonged low-salinity adaptation evaluated in this study was evidently insufficient to elicit a pronounced lysozyme response. A similar trend was noted by Novriadi *et al*. (2025), where non-infectious variables like dietary digestibility enhancers failed to alter lysozyme activity in *L. vannamei*. Taken together, these findings underscore that lysozyme activity in shrimp is fundamentally responsive to infectious stimuli rather than standalone abiotic stressors. Maintaining this baseline lysozyme activity under low-salinity conditions indicates that this humoral component is preserved at a basal level, whereas cellular, hemocyte-related responses appear far more susceptible to salinity-induced physiological stress.

Water quality parameters measured during the experiment are presented in Table 1 below. Temperature, salinity, and pH measurements were taken in situ, while dissolved oxygen and TAN were measured *ex-situ*.

Water quality parameters remained within acceptable ranges according to Kementerian Kelautan dan Perikanan (KKP, 2016) for *L.*

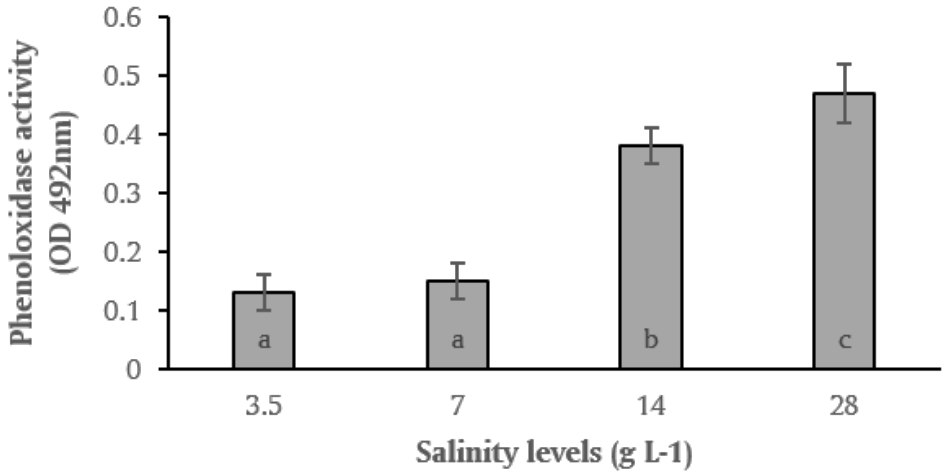


Figure 5. Phenoloxidase (PO) activity of *L. vannamei* juveniles under different salinity levels over 14 days of observation (mean ± SD, n = 3). Different letters indicate significant differences among treatments ($p < 0.05$)

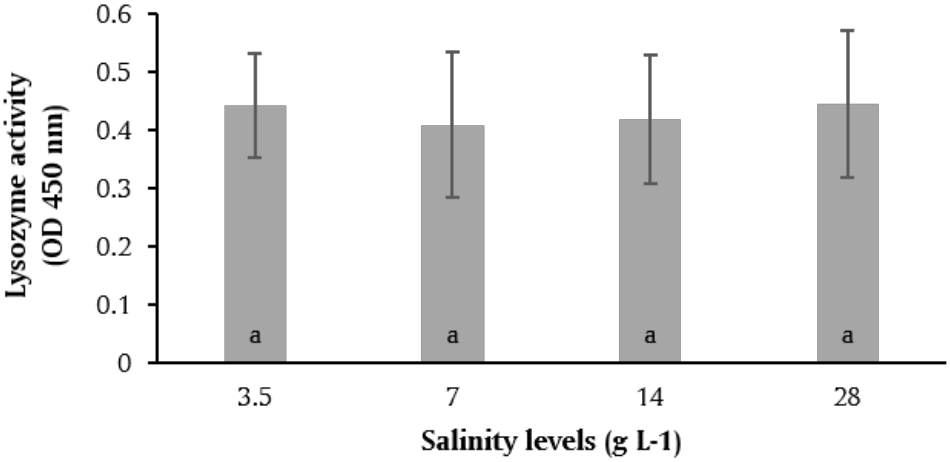


Figure 6. Lysozyme activity of *L. vannamei* juveniles under different salinity levels over 14 days of observation (mean ± SD, n = 3). No significant differences were observed among treatments ($p > 0.05$)

Table 1. Variations of water quality parameters in the rearing tanks of *L. vannamei* juveniles subjected to different salinity treatments for 14 days

Parameters	Range	References
Temperature (^oC)	27-28	27 (KKP, 2016)
Salinity (g L^{-1})	3.5-28	26 - 32 (KKP, 2016)
Dissolved oxygen (mg L^{-1})	5.4-6.5	>4.0 (KKP, 2016)
pH	6.9-7.9	7.5 – 8.5 (KKP, 2016)
Total ammonia nitrogen (mg L^{-1})	0.02-0.10	<0.1 (KKP, 2016)

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vannamei culture throughout the experiment, indicating that the observed physiological and immune responses were primarily associated with the salinity treatment. The observed suppression of several immune-related parameters suggests a physiological trade-off between osmoregulation and immune function under reduced salinity conditions. Under osmotic stress, shrimp must allocate considerable metabolic energy to maintain ionic and osmotic balance, potentially reducing the energy available for immune defense mechanisms. This condition may consequently compromise shrimp resistance to opportunistic pathogens and increase susceptibility to disease outbreaks in low-salinity culture systems. Similar concerns have recently been highlighted by Chuchird *et al*. (2024), who reported mass mortality associated with bacterial infection in low-salinity shrimp farming systems.

The present findings therefore emphasize the importance of adaptive management strategies to support shrimp health and maintain culture performance under low-salinity conditions. Several approaches have shown promise for salinity-induced stress in shrimp aquaculture. Dietary supplementation with β-glucan combined with probiotics has been reported to improve immune responses and energy metabolism under low-salinity stress conditions (Chang *et al*., 2025). In addition, supplementation with organic minerals combined with synbiotics may enhance physiological performance and stress tolerance in shrimp cultured in reduced-salinity environments (Barbosa *et al*., 2025), where ion balance may be disrupted (Boyd, 2020). Biofloc technology (BFT) systems have also demonstrated potential to improve water quality stability and support shrimp performance under low-salinity culture conditions (Al-Subiai *et al*., 2026).

Overall, the present study demonstrated that low-salinity culture conditions may adversely affect the survival and immune competence of *L. vannamei*. Therefore, successful low-salinity culture of this species requires not only physiological adaptation of the shrimp but also appropriate culture management strategies to minimize osmotic stress and maintain immune performance.

CONCLUSION

The present study confirms the hypothesis that prolonged exposure to low-salinity conditions will exceed the osmoregulatory capacity of *L. vannamei* juveniles. This condition induces a physiological trade-off that significantly suppresses critical immune-related parameters and reduces overall survival. In this study, we establish that a salinity of 7 g L^{-1} as a critical physiological threshold; cultivating juvenile shrimp below this level without adequate support poses severe health and mortality risks. Therefore, future studies should focus on evaluating adaptive management interventions such as dietary supplementation with probiotics, immune stimulants, organic minerals, or the implementation of biofloc technology (BFT) to mitigate osmotic stress and enhance the immune resilience of shrimp reared in suboptimal hyposaline environments.

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AUTHOR CONTRIBUTION

R: Conceptualization, supervision, methodology, validation, writing – review & editing. DRAR: Investigation, visualization, formal analysis, writing – original draft. W: Supervision, validation, writing – review & editing. APFA: Writing – review & editing. MAK: Writing – review & editing. APF: Formal analysis, data curation.

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DECLARATION OF COMPETING INTEREST AND USE OF GENERATIVE AI

The authors declare no competing interests. During the preparation of this work, the authors used Gemini in order to refine the academic tone, improve the structural flow of the manuscript, and assist in copyediting. The authors reviewed and edited the content of the created material as needed and take full responsibility for the content of the publication.

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