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The Effect of Temperature on Survival Rate of White leg Shrimp (*Litopenaeus vannamei*) Infected with Infectious Myonecrosis Virus

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ABSTRACT

Infectious myonecrosis virus (IMNV) is a significant pathogen in shrimp aquaculture, causing high mortality. Environmental factors, particularly temperature fluctuations, are known to trigger IMNV outbreaks. The present study aimed to determine the effects of hyperthermia on the survival rate and incubation period of whiteleg shrimp (*Litopenaeus vannamei*) infected with IMNV. The present study utilized a completely randomized design with five treatment groups and three replications. Each replication consisted of 15 shrimp, with an average weight of 9.19 ± 0.58 grams, reared in 100 L plastic containers filled with seawater at 26 ppt salinity. The treatment groups consisted of shrimp infected with IMNV via intramuscular injection and reared at temperatures of 30°C (S30, positive control group), 31°C (S31), 32°C (S32), and 33°C (S33). Additionally, a negative control group of non-infected shrimp was reared at 30°C (Sk30). The observation period lasted 10 days post-infection. The present results demonstrated that hyperthermia significantly impacted shrimp mortality and viral incubation. The highest survival rate was observed in the control group at 97.78%. Among the infected groups, the highest survival rates were recorded in S30 (51.11%) and S31 (48.89%), whereas the lowest were in S32 (28.89%) and S33 (24.44%). In the S33 treatment, higher temperatures reduced the incubation period to 2 days, whereas in the S30 treatment, it lasted for 5 days. The current findings indicated that water temperatures of 32°C and 33°C act as environmental stressors, significantly reducing IMNV incubation period and increasing the risk of mortality in *Litopenaeus vannamei* shrimp.

Keywords: Hyperthermia, Infectious myonecrosis virus, Incubation period, Survival rate

INTRODUCTION

Indonesian shrimp culture has experienced rapid growth, with national production reaching 851.6 thousand tons in 2020 (KKP, 2022). The government has planned to increase shrimp production to 2 million tons by 2024. Among cultivated species, the whiteleg shrimp (*Litopenaeus vannamei*) is the dominant commodity, accounting for approximately 65% of total national production (Purnomo et al., 2018; Palupi et al., 2022; 2023). However, increasing shrimp production is currently hindered by the emergence of infectious myonecrosis virus (IMNV; Baladrat et al., 2022). Infectious myonecrosis virus was first reported in Brazil in 2002 (Miller and Zachary, 2017), followed by its detection in Indonesia in 2006 (Naim et al., 2014). The IMNV outbreak in Indonesia was attributed to the import of whiteleg shrimp from Brazil. The entry of whiteleg shrimp in Indonesia began at the end of 1999 (Sugama et al., 2006). Infectious myonecrosis virus is a double-stranded RNA virus that belongs to the family Totiviridae (Arulmoorthy et al., 2020). In early 2006, IMNV occurred during the 70-90-day period of shrimp rearing (Sunarto and Naim, 2016). Infectious myonecrosis virus outbreaks typically occur between days 30 and 90 of the culture period, often leading to cumulative mortality rates of up to 70%. Beyond direct stock losses, the infection severely compromises production efficiency by increasing the feed conversion ratio (FCR) from a baseline of 1.5 to as high as 4.4, thereby causing significant economic losses (Lightner et al., 2004; Larasati et al., 2021).

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Infectious myonecrosis virus is recognized as a major threat to global *Penaeus vannamei* aquaculture, with outbreaks frequently triggered by environmental stressors, such as temperature and salinity fluctuations, which compromise host immunity and lead to acute mortality (Poulos et al., 2006). A focused investigation into the role of thermal stress is necessary, as recent findings indicated that temperature fluctuations can directly affect the severity and progression of the infection (Cirino et al., 2024). High-temperature stress causes intestinal mucosal damage, immune suppression, and increased susceptibility to disease in whiteleg shrimp (Duan et al., 2025). Shrimp measuring less than 5 grams grow fast at a temperature of 30ºC, while shrimps measuring more than 16 grams grow optimally at a temperature of 27ºC (Wyban et al., 1995). Heat stress at 33°C induces metabolic shifts and remodeling in the hepatopancreas, leading to oxidative damage and disruption of immune homeostasis, thereby rendering the shrimp more susceptible to viral replication (Duan et al., 2025). An optimal temperature range for production is 25°C-30°C, as indicated by metabolic rate measurements using standard metabolic rate. Temperatures above 30°C tend to increase stress in shrimp (Harlina et al., 2022; Kir et al., 2023). This hyperthermic condition accelerates physiological processes, leading to excessive oxygen consumption and rapid depletion of energy reserves. Consequently, energy that should be allocated for growth and immune response is diverted solely to maintain homeostasis and survival (Wang et al., 2019).

Clinical signs of IMNV infection in shrimp include alterations in abdominal muscle tissue, which become transparent and white (Hidayat et al., 2019). In some cases, the tail and the last muscle segment turn red (Prasad et al., 2017). Similar to other viral infections, shrimp appear weak and exhibit decreased appetite. The IMNV epidemic appears to be influenced by factors such as feed quality, net use, salinity fluctuations, and extreme temperature changes. However, detailed data on the relationship between these predisposing factors and IMNV remains limited (Lee et al., 2022). Specifically, quantitative data on the impact of high-temperature stress on mortality rates in whiteleg shrimp are lacking. Therefore, the present study was conducted to determine the effect of high temperature on the survival rate and incubation period of *Litopenaeus vannamei* infected with IMNV.

MATERIALS AND METHODS

Ethical approval

The current study was carried out in accordance with the guidelines of the Faculty of Aquaculture Technology at the Jakarta Technical University of Fisheries, Jakarta, Indonesia. All procedures adhered to standard protocols for laboratory and field testing. Daily analysis of the test biota involved physical observations, with food supplied according to each treatment group.

Experimental design

The present study was conducted as an experimental laboratory challenge test using a completely randomized design (CRD). The experiment included five treatment groups with three replications. The experimental design consisted of five treatment groups, including a negative control comprising uninfected whiteleg shrimp reared at 30°C (SK30), a positive control of IMNV-infected shrimp maintained at 30°C (S30), and three infected groups subjected to elevated temperatures of 31°C (S31), 32°C (S32), and 33°C (S33). The shrimp for this study were obtained from the Fish Health and Environmental Laboratory in Serang, Indonesia. A total of 225 shrimp were used; each tank was stocked with 15 juvenile shrimp, with an average weight of 9.19 ± 0.58 g. The shrimp were acclimated in a maintenance container for one week prior to being subjected to treatment according to the experimental protocol. During the adaptation and treatment periods, the shrimp were provided with commercial feed (PT Suri Tani Pemuka Japfa Comfeed, Indonesia), characterized by a particle size and nutritional composition ranging from 1.6 to 1.8 mm, with a crude protein content of 30%, crude fat at 5%, crude fiber at 6%, ash at 13%, moisture at 12%, and a metabolizable energy content of 3,000 kcal/kg. The feed was administered daily, amounting to 5% of the biomass, with feeding occurring at 09:00 a.m., 15:30, and 21:00.

The experimental units included 15 plastic tanks, each with a volume of 100 liters, equipped with individual aeration lines to maintain dissolved oxygen (DO) levels above 5 mg/L. To maintain stable water temperatures for the specific treatments, 100-watt submersible heaters equipped with thermostats were installed in each treatment tank (Wang et al., 2019).

Preparation of maintenance container

The maintenance container used is a 100 L plastic box. A total of 15 units of containers were disinfected using 50 ppm hypochlorite solution, rinsed with fresh water, and dried. The container was subsequently filled with aerated seawater at 30 ppt salinity. Water quality was maintained by siphoning and replacing up to 10% of the water daily (Widanarni et al., 2016).

Virus reinfection

The IMNV stock was sourced from the Fish Health and Environmental Laboratory collection. A 0.1 mL portion of IMNV virus stock was injected into whiteleg shrimp. The shrimp's abdominal tissue was cleaned, and the carapace was removed. Subsequently, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed on the tissue to quantify viral copies, which were then used to prepare the virus inoculum.

Virus inoculum and procedure

A total of 100 g of shrimp abdominal muscle tissue was infected with IMNV (without carapace), crushed with 300 mL of Tris-HCl NaCl buffer (0.4 M NaCl and 20 mM Tris-HCl, pH 7.4). The tissue in the TN buffer was diluted 10 times with a 2% NaCl solution. The inoculum preparation was then centrifuged at 3,000 rpm for 25 minutes at 40°C. The supernatant solution was collected, centrifuged again at 14,000 g for 20 minutes at 40°C. Then, the supernatant was collected and filtered through a 0.22 µm syringe filter. The inoculum stock was then stored in a deep freezer at -80°C (da Silva et al., 2015). Shrimp were infected with 0.1 mL of IMNV inoculum via intramuscular injection. The injection was conducted in the third segment of the dorsal abdomen using a 1 ml sterile syringe with a needle size of 25 G. After the injection, the shrimp were reared by feeding 5% of the biomass (Feijó et al., 2015).

Post-infection observation procedures

Shrimp were observed every four hours for eight days. Survival rate and clinical signs were observed daily. In addition, water, hemolymph, and organ samples were taken during the study. Water samples were used for testing water quality. Hemolymph and muscle tissue samples were used for qRT-PCR testing. Confirmation of IMNV infection was performed by qRT-PCR in accordance with SNI 7662.1.2011 (Hanggono and Junaidi, 2015). Muscle tissue and lymphoid organs were fixed using Davidson's solution for histopathological testing (Baladrat et al., 2022). The histopathological test method follows SNI 7304.2018 (Indonesian National Standard, 2018). Strict biosecurity protocols were implemented at the Fish Health and Environmental Laboratory in Serang, Indonesia, to prevent the release of the virus into the environment. The challenge test was conducted in a restricted-access wet laboratory.

Survival rate

The survival rate indicated the percentage of live shrimps compared to the initial number at the start of the study. The survival rate was calculated based on the following formula (Tani et al., 2025).

$$\text{Survival rate (\%)} = \frac{\text{Nt (Number of shrimp alive at end of the study)}}{\text{No (Number of shrimp released at the start of study)}} \times 100$$

Histopathological description

In addition to the survival rate, the present study observed clinical signs, including infection and physical changes in shrimp. Based on the severity of the infection, IMNV lesions were grouped into four levels (Jha et al., 2021; Table 1). Histopathological data were obtained by observing changes in muscle tissue and lymphoid organs, which were the targets of the IMNV infection (Melena et al., 2012). Viral confirmation was performed using reverse transcriptase PCR (RT-PCR). Infection in the IMNV-positive sample groups was confirmed by the detection of a specific 328 bp amplification product (band). Conversely, the absence of this diagnostic band indicated that the samples were negative for IMNV.

Water quality parameters

Daily water quality measurements were conducted at 6 a.m. and 5 p.m. The water quality measurements, including temperature, salinity, DO, and pH, were conducted *in situ* (BSN, 2014). Meanwhile, nitrate, total ammonia nitrogen (TAN), f (TOM), and hydrogen sulfide (H_2S) were measured *ex situ*. For *ex situ* measurements, 250 mL of water was taken (APHA, 2017). The parameters and methods for measuring water quality are shown in Table 2.

Data analysis

Survival rate data were analyzed using a one-way ANOVA with a 95% confidence level of p value less than 5% ($p < 0.05$). The software used is IBM SPSS version 22.0. Meanwhile, data on the incubation period and the distribution of clinical signs onset were analyzed using the Kaplan-Meier method to estimate the cumulative probability of signs appearance over the observation period. The PCR test results and histopathology were analyzed descriptively.

Table 1. Clinical signs and lesion severity observed in Pacific white shrimp (*Litopenaeus vannamei*) infected with infectious myonecrosis virus during the study (Indonesia, 2025)

Degree of lesion	Symbol	Clinical signs
Level 1	*	Minor change in muscle color, appearing whitish or gray in limited areas (focal lesions). There was a decrease in appetite, but no death was found.
Level 2	**	The muscle color appeared less transparent, with a whiter tone compared to level 1. The area of abdominal necrosis was expanding.
Level 3	***	The muscle's white color (not transparent) was more pronounced and concentrated in the upper and lateral parts of the abdomen, including the basal pleopod. White color reached the cephalothorax. The shrimp was weak and had lost its appetite.
Level 4	****	A milky-white color was found in almost all parts of the abdomen. A reddish color in the tail and last segment was observed. The shrimp was weak, and death occurred.

Table 2. Units and methods of water quality parameter measurement

Parameters	Unit	Tools/Methods
Daily checking (morning and evening)		
- Temperature	^oC	Thermometer
- Salinity	Ppt	Refractometer
- DO	mg/L	DO-meter
- pH	-	pH-meter
Weekly checking		
- Nitrite	mg/L	Spectrophotometry (SNI 06-6989.9-2004)
- TOM	mg/L	Titrimetry (SNI 06-6989.22-2004)
- TAN	mg/L	Spectrophotometry (SNI 06-6989.30-2005)
- H_2S	mg/L	Colorimetry (SNI 6989.70:2009)

DO: Dissolved oxygen, TOM: Total organic matter, TAN: Total ammonia nitrogen, H_2S: Hydrogen sulfide, ppt: Parts per thousand.

RESULTS

Survival rate

The survival rate in the control group (Sk30) at 97.78% differed significantly from those of all IMNV-infected groups (S30, S31, S32, and S33). The survival rates in the S30 and S31 were 51.11% and 48.89%, respectively. There was no significant difference between the S30 and S31 treatments ($p > 0.05$). There was no significant difference between the S32 (28.89%) and S33 (24.44%) treatments ($p > 0.05$). The S33 was significantly different from the control and low hyperthermia groups (S30 and S31; $p < 0.05$). The current results demonstrated a distinct inverse relationship between water temperature and survival rate in IMNV-infected shrimps. Hyperthermia at 32°C and 33°C significantly increased the risk of death, suggesting that temperature served as a critical environmental stressor that exacerbated IMNV mortality ($p < 0.05$; Figure 1).

Incubation period of infectious myonecrosis virus

The current results indicated that infected shrimp with IMNV exhibited clinical signs ranging from level 1 to 4. Clinical signs and behavioral changes appeared simultaneously. In general, clinical signs start with a loss of appetite and are followed by physical changes. However, the shrimp exhibited physical changes without a decrease in appetite. Clinical signs range from loss of appetite to abdominal discoloration. The cumulative percentage analysis indicated that 50% of the clinical signs were observed in the S33, S32, S31, and S30 treatment groups on days 5, 6, 8, and 10, respectively (Figure 2).

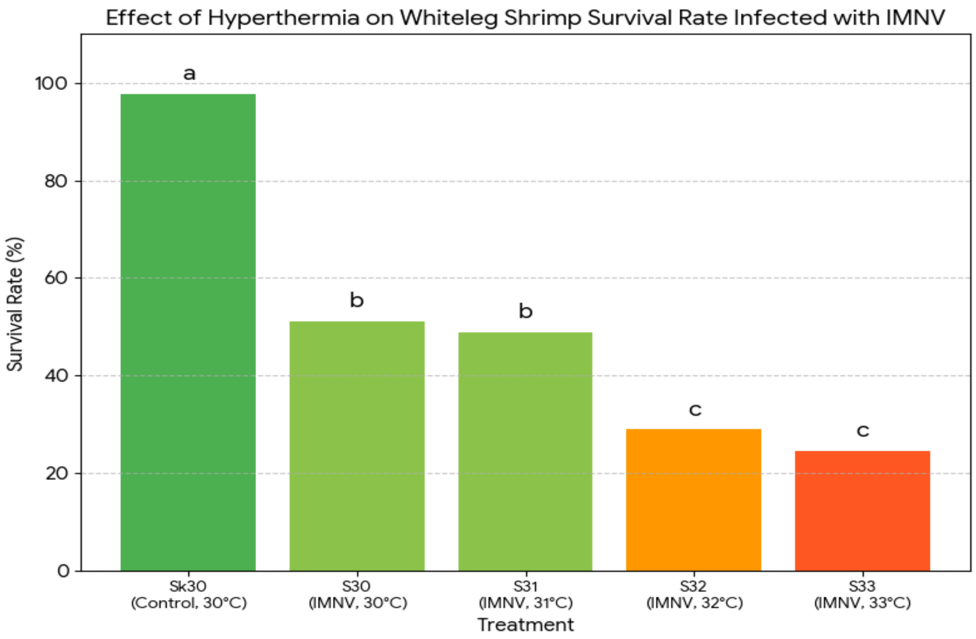

Figure 1. The survival rate of whiteleg shrimp (*Litopenaeus vannamei*) infected with infectious myonecrosis virus (IMNV) and reared at different temperatures after 10 days of maintenance. S30: IMNV at 30°C, S31: IMNV at 31°C, S32: IMNV at 32°C, S33: IMNV at 33°C, and SK30: Control with no IMNV infection at 30°C. [a,b,c] Different superscript letters indicated a significant difference among groups ($p < 0.05$)

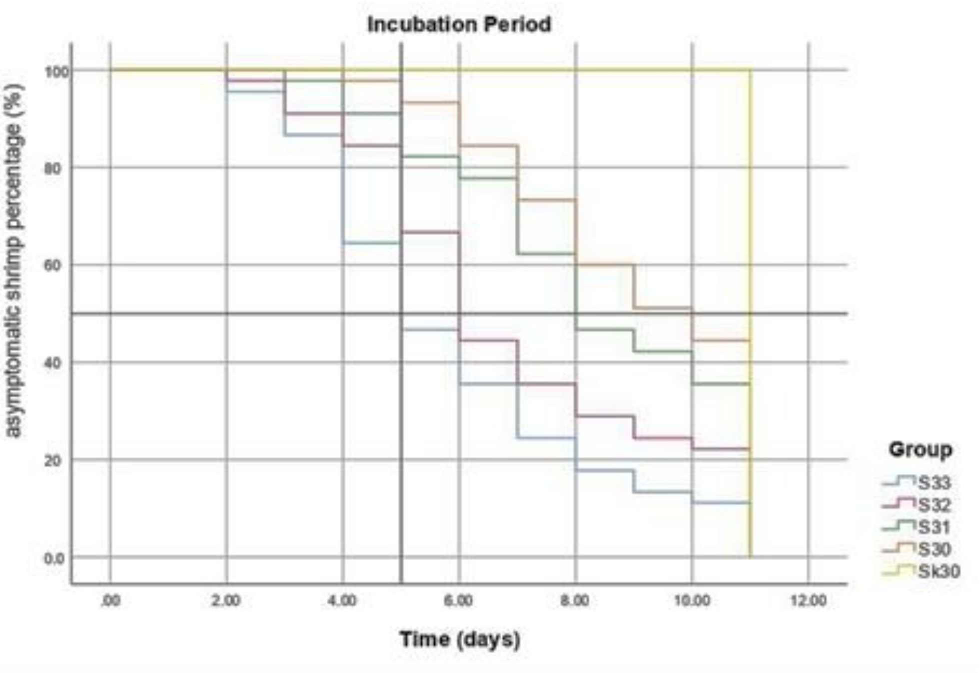

Figure 2. Kaplan Meier results analysis based on observations of clinical signs of whiteleg shrimp infected with infectious myonecrosis virus and reared at different temperatures. S30: IMNV at 30°C, S31: IMNV at 31°C, S32: IMNV at 32°C, S33: IMNV at 33°C, and SK30: Control with no IMNV infection at 30°C

Water quality test

The DO concentrations in *Litopenaeus vannamei* culture water ranged from 6.01 to 6.37 mg/L, which were within the reference standard of > 4 mg/L. The salinity measurements ranged from 26.0 to 26.9 ppt, which were within the optimal range for whiteleg shrimp of 20-45 ppt. The pH values ranged from 7.24 to 8.51, within the optimal range of 6-9. nitrite levels ranged from 0.016 to 0.576 mg/L, within the safety threshold of < 1 mg/L. The results of the TAN levels in *Litopenaeus vannamei* culture water ranged from 0.003 to 0.751 mg/L. The established threshold for TAN in intensive farming is less than 0.05 mg/L. The H_2S concentration ranged from 0.01 to 0.143 mg/L, all of which were compared to the reference standard of less than 0.05 mg/L. Furthermore, TOM measurements ranged from 33.50 to 117.55 mg/L, which were within the standard range for intensive farming (<150 mg/L). Overall, the water quality parameters generally remained within the optimal range for the species across all treatment groups (Table 3).

Table 3. Water quality parameters at different temperatures in whiteleg shrimp (*Litopenaeus vannamei*)

Parameter \ Treatment	S30	S31	S32	S33	Sk30	Standard
Temperature (°C)	30.0 ± 0.10	31.0 ± 0.09	32.0 ± 0.07	33.0 ± 0.06	30.0 ± 0.06	20-32 (BSN, 2015)
DO (mg/L)	6.31 ± 0.50	6.26 ± 0.45	6.01 ± 0.54	6.13 ± 0.55	6.37 ± 41	> 4 (Boyd, 1989)
Salinity (ppt)	26.9 ± 0.40	26 ± 0.62	26.0 ± 0.54	26.1 ± 0.36	26.0 ± 0.22	20-45 (Chong-Robles et al., 2014)
pH	7.94 - 8.44	7.24 - 8.51	7.94 - 8.47	7.88 - 8.49	7.6 - 8.43	6-9 (Boyd, 1989)
Nitrite (mg/L)	0.022 - 0.182	0.025 - 0.576	0.02 - 0.314	0.029 - 0.471	0.016 - 0.177	< 1 (BSN, 2015)
TAN (mg/L)	0.023 - 0.217	0.037 - 0.426	0.048 - 0.751	0.003 - 0.066	0.017 - 0.144	< 0.05 (BSN, 2015)
H_2S (mg/L)	0.01 - 0.057	0.014 - 0.143	0.019 - 0.107	0.011 - 0.091	0.011 - 0.091	< 0.05 (Boyd, 1989)
TOM (mg/L)	33.50 - 117.55	43.76 - 104.28	52.46 - 104.91	49.93 - 115.02	33.50 - 117.55	< 150 (Ariadi et al., 2019)

S30: IMNV at 30°C, S31: IMNV at 31°C, S32: IMNV at 32°C, S33: IMNV at 33°C, and SK30: Control with no IMNV infection at 30 °C. DO: Dissolved oxygen, TAN: Total ammonia nitrogen, TOM: Total organic matter

Histopathological description

Histopathological examination confirmed severe tissue damage in the IMNV-infected groups (Figure 3). The abdominal muscle tissue exhibited coagulative necrosis, characterized by the loss of normal muscle structure and significant hemocytic infiltration. Intracellular inclusion bodies were widely distributed within the muscle fibers, while advanced tissue damage was marked by fibrosis and liquefaction necrosis. Furthermore, the lymphoid organs exhibited distinct hypertrophy, driven by the extensive formation of lymphoid organ spheroids (LOS). The arrows point to the spherical accumulations of cells (spheroids) within the lymphoid tissue, which replaced normal tissue structure. Histopathological analysis demonstrated that the structure of the muscles and lymphoid organs has changed in the shrimps (Chávez-Sánchez et al., 2020).

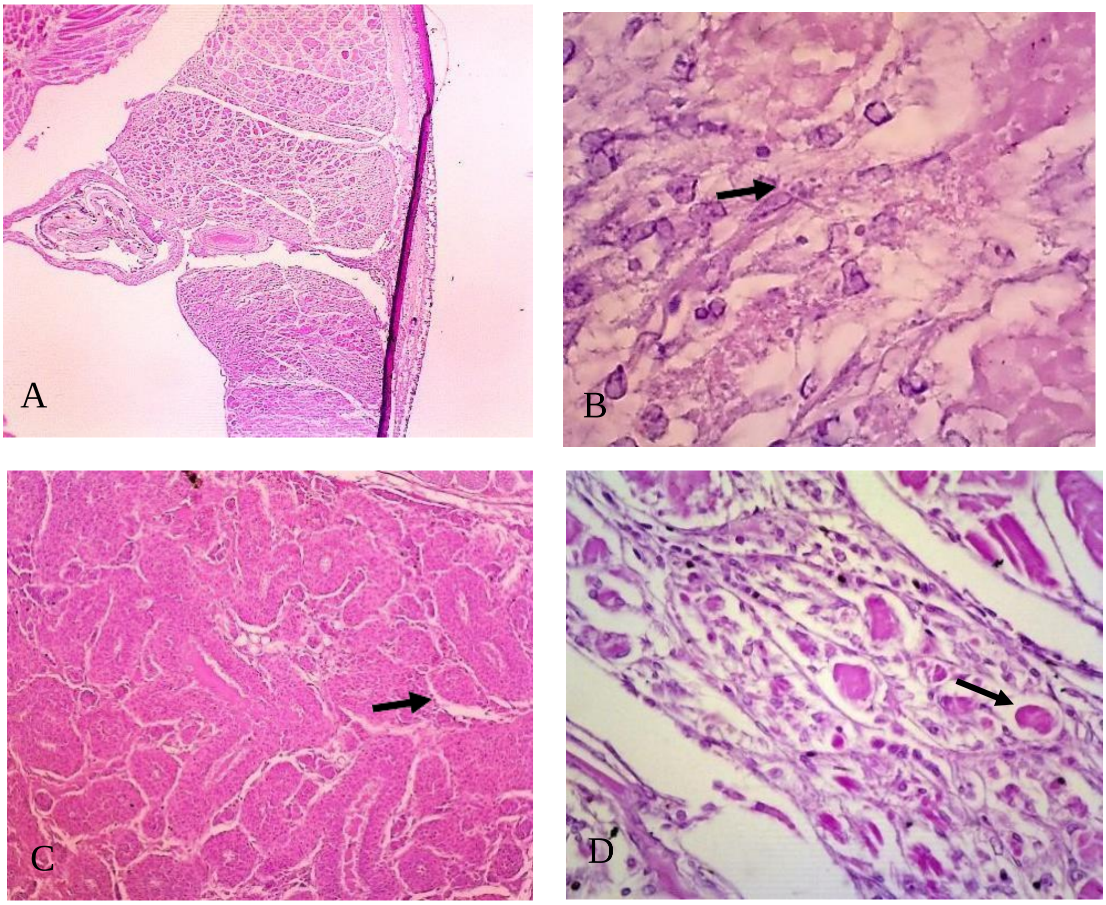

Figure 3. Histopathological view of muscles and the lymphoid organ in whiteleg shrimp infected with infectious myonecrosis virus. **A:** Coagulative necrosis with hemocyte infiltration of muscle tissue. **B:** Muscle tissue inclusion bodies. **C:** Spheroid organ lymphoid formation (LOS, black arrow). **D:** Fibrosis and liquefaction necrosis.

qRT-PCR confirmation test

The RT-PCR was then performed on shrimp exhibiting clinical signs or mortality to confirm the presence of IMNV. The RT-PCR results indicated that the treatment groups (S30, S31, S32, and S33) were positive for the presence of IMNV. Meanwhile, negative IMNV results were observed in the Sk30 treatment group. A positive IMNV result was observed by the presence of a band at 328 base pairs (bp; Figure 4).

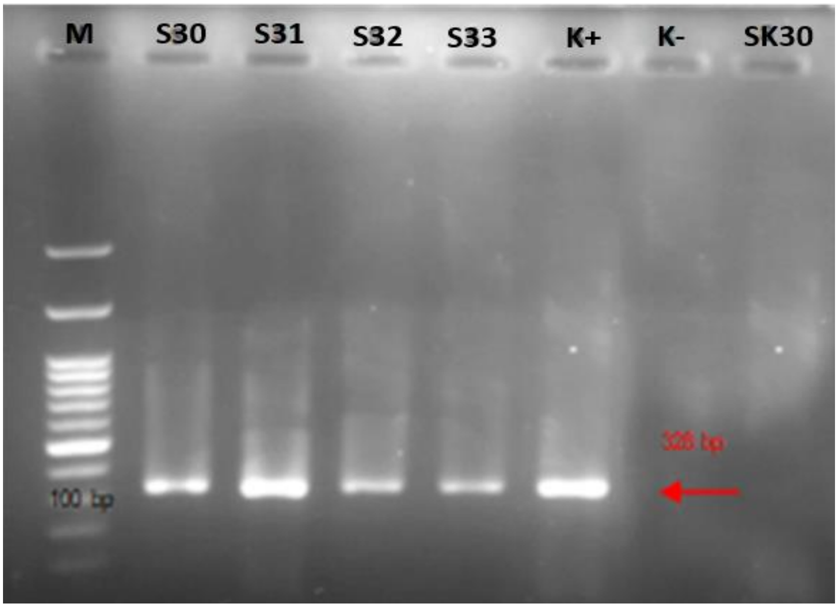

Figure 4. qRT-PCR results of whiteleg shrimp after the IMNV challenge test. M: Marker (100 bp) DNA ladder used as a size reference, S30: Maintenance temperature 30°C, S31: Maintenance temperature 31°C, S32: Maintenance temperature 32°C, S33: Maintenance temperature 33°C, K(+): Positive control, K(-): Negative control, Sk30 Negative control treatment, 328 base pairs red arrow confirms a positive result for infectious myonecrosis virus.

DISCUSSION

Among the shrimps infected with IMNV, the lowest survival rate was observed in the S33 treatment group (24.44%). Meanwhile, the highest survival rate was observed in the S30 at 51.11%. All shrimp deaths were followed by necrotic lesions in the abdominal muscles. Hyperthermia increased the shrimp's response to feed. Therefore, this condition can lead to increased fecal output and initiate ammonia production (Xu et al., 2019). Elevated ammonia levels can remarkably decrease hemocyte count and phenoloxidase activity in shrimp. A decrease in immune response parameters can reduce phagocytosis of pathogens (Ambarsari and Satyantini, 2020). Data processing of the present results indicated that ammonia was associated with immune response parameters.

The Kaplan Meier curve can compare the distribution of the incidence of several groups receiving different treatments (Abou-Shaara, 2018). Clinical signs of whiteleg shrimp infected with IMNV were observed on the second day post-infection. Meanwhile, new deaths occurred on the fourth day after infection.

In general, shrimp infected with IMNV appeared weak, exhibited decreased appetite, and showed discoloration of the abdominal muscles. Decreased appetite is a nonspecific sign often observed in response to different infectious and noninfectious stressors (Kooloth Valappil et al., 2025). In this study, hyperthermia considerably reduced the incubation period. Clinical signs appeared as early as day 2 at 33°C, while at 30°C, the incubation period extended to 5 days. The physiological costs of thermal stress likely led to rapid disease progression and decreased appetite (Wang et al., 2019). High temperatures trigger a metabolic shift in the hepatopancreas, leading to oxidative damage and a breakdown of immune homeostasis, thereby increasing vulnerability to viral replication (Duan et al., 2025). Additionally, in the presence of environmental stressors such as temperature changes, shrimp should shift energy normally used for immunity and growth to preserve homeostasis (Nurhudah et al., 2024). Hyperthermia increases energy expenditure, leading to depletion of lipid and glycogen reserves, whereas hypothermia results in minimal mobilization of energy stores during recovery (Barajas-sandoval et al., 2024).

In general, water quality remained within the optimal range. Water quality conditions influence shrimp immunity (Iunes et al., 2021). Hyperthermia can damage intestinal mucosal structure, suppress immunity, and make shrimps more susceptible to disease. Water quality, including pH value of 7.6 to 8.51, remained within the optimal range for the species across all treatment groups. In other studies, the pH level of water ranged from 7.5 to 7.6 in the morning and from 7.9 to 8.2 in the afternoon. Overall, the water pH was within the optimal range, and fluctuations were stable

(Pratiwi et al., 2024). Shrimp reared at 25 ppt salinity and transferred to 5 ppt and 15 ppt within 12 hours demonstrated reduced immunity and were more susceptible to bacterial infections (Aranguren Caro et al., 2021). Changes in salinity from 25 ppt to 15 ppt could have reduced shrimp immunity (Pan et al., 2005). Total ammonia nitrogen can reduce shrimp immune levels (Mahasri et al., 2019; Deriyanti et al., 2021; Fitriadi et al., 2023). Under hypoxic conditions (DO level less than 3.5 mg/L), shrimp immunity decreases, as indicated by phenoloxidase and total haemocyte counts (Cheng et al., 2005). Chronic exposure to nitrite levels of 6.67 ppm can inhibit growth and reduce immunity by disrupting gut microbial homeostasis. Acute exposure to sulfides (5 mg/L) can damage gills and reduce shrimp immune enzymes within 12 to 72 hours (Duan et al., 2018).

The abdominal muscles of the whiteleg shrimp lost transparency or became white due to necrosis. The lower abdominal and tail muscles are often severely injured. This condition occurs because the muscle mass in the abdominal and tail regions are larger than in other areas (Sunarto and Naim, 2016; Mahasri et al., 2018). The onset of muscle necrosis typically becomes visible by the third day post-infection (Wan et al., 2023) and progresses to extensive necrosis throughout the muscle tissue by the fifth day (Chen et al., 2017). In addition to muscle, the target organs of IMNV are hemocytes and lymphoid organs (Feijó et al., 2015). Alterations in shrimp muscle structure are evident in the occurrence of *Penaeus Vannamei Nodavirus* (*PvNV*) and *Macrobrachium rosenbergii Nodavirus* (*MrNV*; Senapin et al., 2013) and in muscle cramp syndrome (Sunarto and Naim, 2016).

Histopathological examination of IMNV infection revealed coagulative necrosis, hemocyte infiltration, and inclusion body formation (Lee et al., 2022). Meanwhile, in the lymphoid tissue, LOS formation was observed, leading to lymphoid hypertrophy. In shrimp, LOS formation is linked to viral infection (Melena et al., 2012). Consequently, this extensive cellular accumulation led to hypertrophy of lymphoid tissue, a characteristic sign of chronic viral infection (Prasad et al., 2017). The *neprocomplex* (antennal gland) is reported to be the site of pathogens' entry in shrimp during the molting process (de Gryse et al., 2020).

CONCLUSION

The current results indicated that hyperthermia decreased the survival rate in whiteleg shrimp infected with IMNV. Whiteleg shrimp infected with IMNV, reared at 32°C and 33°C, had a higher risk of death than those reared at 30°C and 31°C. In addition, hyperthermic conditions reduced the incubation period of IMNV. For future studies, it is advisable to examine the molecular mechanisms underlying immune suppression during thermal stress, with a focus on the expression of stress-related genes and heat shock proteins. Furthermore, field investigations on mitigation strategies, including the use of immunostimulants or water-temperature management protocols in intensive pond systems, are essential to reduce economic losses resulting from IMNV outbreaks.

DECLARATIONS

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Availability of data and materials

All relevant data generated during the study are included in this published article and are available upon reasonable request from the corresponding author.

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Authors' contributions

Irvan Firman Syah Za conducted data collection and wrote the original manuscript. Azam Bachur Zaidy did the research execution and data collection. Ketut Sugama and Soebhakti Hasan assisted in data analysis, administration, and conceptualization. Ren Fitriadi, Muhammad Ikhwan Ihtifazhuddin, Sata Yoshida Srie Rahayu, I Made Dedi Mahariawan, and kamaruddin Interpretation of data, wrote the draft, and prepared the article. All authors read and confirmed the final edition of the manuscript.

Ethical considerations

All authors have carefully reviewed the manuscript to ensure there are no ethical concerns, including plagiarism, research misconduct, data fabrication or falsification, and redundant publication. No AI tools were used during the preparation of the present study.

Competing interests

The authors have not declared any conflict of interest.

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